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VOLUME 9 JULY, 1933 NUMBER 1

THE BIOLOGY OF *PODISUS SERIEVENTRIS* UHLER, IN CAPE BRETON, NOVA SCOTIA[1]

BY M. L. PREBBLE[2]

Abstract

The biology of *Podisus serieventris* Uhler, and its role in an outbreak of the black-headed budworm, *Peronea variana* Fernald, in Cape Breton, N.S., are described from studies carried on in 1930 and 1931. There is but one complete generation of *Podisus* a year, and adults of both sexes hibernate. The eggs are laid in late June, July and early August, the incubation period ranging between 10 and 15 days. There are five nymphal stages, requiring about 45 days, on the average, for the attainment of the adult condition. In these respects particularly, the life history of *Podisus serieventris* in Cape Breton differs from its life history in Massachusetts, where four nymphal stages and three annual generations and the hibernation of females only, have been reported.

The species conforms satisfactorily to Dyar's Law, the average growth ratio of individuals studied in 1931 being about 1.28. The first-stage nymphs feed on unhatched eggs of their own species, and upon the juices of coniferous and deciduous foliage, but were not induced to feed upon small caterpillars. Nymphs were able to complete the first instar on a purely vegetable diet, but died before the second moult when the same diet was continued. Older nymphs, fed for some time on animal food, were not able to attain the adult condition when supplied with plant food alone. This indicates the dependence of the species upon animal food; the food consumption of the various stages is briefly summarized. Evidence is presented which suggests the utilization, by *Podisus*, of a toxic secretion in overcoming their prey.

The rather limited value of *Podisus* as a control factor in the outbreak of *Peronea variana* in 1930 and 1931 is described. The decline of the *Peronea* population in 1931 caused a corresponding mortality in the *Podisus* population, by starvation.

Introduction

This paper includes the more important biological data which, together with a study of the external anatomy of the adult, were incorporated in a thesis "The biology of *Podisus serieventris* Uhler, with especial reference to its predatory habits," submitted to the Faculty of Graduate Studies and Research, McGill University, in April 1932. The paper deals with the species in Cape Breton Island, N.S., in relation to the black-headed budworm, *Peronea variana* Fernald.

[1] *Manuscript received April 5, 1933.*

Contribution from the Dominion Entomological Laboratory, Fredericton, New Brunswick, Canada. This paper was constructed from a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, Montreal, Canada, in April 1932, in part fulfilment of the requirements for the degree of Master of Science.

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Distribution and Habitat

The predacious pentatomid, *Podisus serieventris* Uhler, is distributed throughout the maritime provinces, Quebec and Ontario, through the New England states to New Jersey and Pennsylvania in the east, also in Illinois and Minnesota, and in Colorado, Wyoming and Montana in the west. It has also been recorded from Vancouver Island. The species is particularly an inhabitant of shade and forest trees. Brief references to the usefulness of *Podisus serieventris*, as an enemy of tree defoliators, occur in the literature; Kirkland (7, 8) studied the species in Massachusetts, where it preys upon the larvae of the gipsy moth.

Severe damage in the balsam-spruce forests of southern Cape Breton, caused by *Peronea variana*, was reported to the Dominion Entomological Branch in July, 1929. The outbreak was investigated in 1930, by Mr. R. E. Balch, entomologist-in-charge, Dominion Entomological Laboratory, Fredericton, N.B., assisted by the writer. Among the factors of control operating against the budworm, *Podisus serieventris* gave promise of some importance. Particular attention was centered on *Podisus* in 1931, to elucidate the interrelations between host and predator.

Methods of Study

Field observations and collections of bugs were made during the course of population studies of *Peronea*. Early in the season, when the insects were very small, trees were felled so that the crown rested on a large cotton mat, 16 ft. square. The limbs were cut off, and carefully examined, and insects which dropped out of the foliage onto the mat were also collected. Later it was found advisable to delimb the standing trees; climbing irons were employed in ascending the trees, and the limbs were lowered down onto the mat by means of stout cord. In this manner, the budworm and stink-bug populations were carefully followed from June till the middle of September. Detailed information on the biology of *Podisus* was obtained by rearing individuals in shell vials from time of hatching until after maturity. A 7 by 9-ft. silk tent, set up at Grand River, Cape Breton, served as a field laboratory.

Identification

Individuals collected at St. Peters, C.B. in 1930, were identified as *Podisus serieventris* Uhler, by Mr. G. S. Walley, Division of Systematic Entomology, Ottawa. Additional specimens, collected at Grand River in 1931, were given the same determination by Dr. H. M. Parshley, Smith College, Massachusetts. The question of authoritative determination is important, for at least three writers have referred to the confusion between *Podisus serieventris* Uhler and *P. maculiventris* (Say.). Van Duzee (20) writes: "Mr. Kirkland reports this (*P. serieventris*) as 'by far the most common representative of the genus' in Massachusetts, but his description seems to refer to the form given here as *maculiventris*." It should be noted, however, that Kirkland had his material identified by Professor Uhler, the author of *serieventris*. Morrill (11) states. "..... whether or not the form known to some as *serieventris* be ultimately

considered as a species distinct from *P. maculiventris*, its habits appear to be the same as those of the latter." Again, Parshley (see Britton, 3) says: "The form described here (as *serieventris*) agrees with Uhler's description and type, but it is not the *serieventris* of some authors. The black spot of the corium appears to be a constant feature and together with the rather blunt pronotal angles and short ventral spine will serve to distinguish it from the other members of the genus." The quotation of Van Duzee is especially relevant, as Kirkland has published, so far as the writer is aware, the only extensive study of *P. serieventris* in America. One inclines to the view that Kirkland's specimens were probably identified correctly. If so, the details in the biology of the species in Cape Breton differ greatly from those recorded for Massachusetts, as described later.

Baker (2) distinguishes between the two species as follows:

P. maculiventris: "Basal spine of abdomen long, extending between hind coxae; lateral angles of pronotum prominent and acute; length 10-12.5 mm.; color greyish-brown."

P. serieventris: "Basal spine of abdomen short, not extending between hind coxae; lateral angles of pronotum blunter."

"Second antennal segment one-third longer than the third; lateral angles of pronotum not prominent; color greyish-brown, corium with a dark spot; length 8-10 mm."

Description

Adult

Professor Uhler's original description (19) was transcribed by Kirkland (7). The specimens taken in Cape Breton agree very well with Uhler's description. The majority of individuals are blackish-gray in color; a few are grayish-brown, with the embolium and distal portion of the corium of a reddish hue. In a few individuals the cephalo-lateral margins of the pronotum are reddish, but in the majority of specimens the margins are yellow. The female bugs are considerably larger than the males. Measurements of a number of adults of both sexes, selected at random from the collection, are shown in Table I.

TABLE I

BODY MEASUREMENTS OF *Podisus serieventris*

Dimension measured	Male adults			Female adults		
	Number measured	Range, mm.	Average, mm.	Number measured	Range, mm.	Average, mm.
Head width	12	1.20– 1.54	1.42	17	1.42– 1.64	1.55
Humeral width	11	4.6 – 5.5	5.0	10	4.9 – 6.3	5.7
Length to tip of abdomen	11	8.5 – 9.9	9.0	10	9.1 –11.4	10.6
Length to apex of hind wing at rest	11	9.5 –11.1	10.3	10	10.2 –12.7	11.7

Egg (Fig. 1)

The eggs are metallic bronze in color, caldron-shaped, tapering slightly toward the posterior pole. In height they range from 1.20 to 1.25 mm., in diameter from 0.90 to 0.96 mm. The circular lid or operculum, with its dense armature of short, stiff, dark spines, covers the anterior pole of the egg. Numerous curved rod-like outgrowths, the chorionic processes, the "seminal cups," or "elongated cup-shaped micropyles" of Leuckart, surround the operculum. They vary in number from 10 to 14, with an average of 12 for 55 eggs examined. Earlier investigators believed that the spermatozoa entered the egg through these chorionic processes. Heidemann (6) states that more recent opinion inclines to the view that they serve to ventilate the interior of the egg. The walls of the egg are ornamented with shorter and more sparsely placed spines. The surface of the egg is nearly bare near the posterior pole.

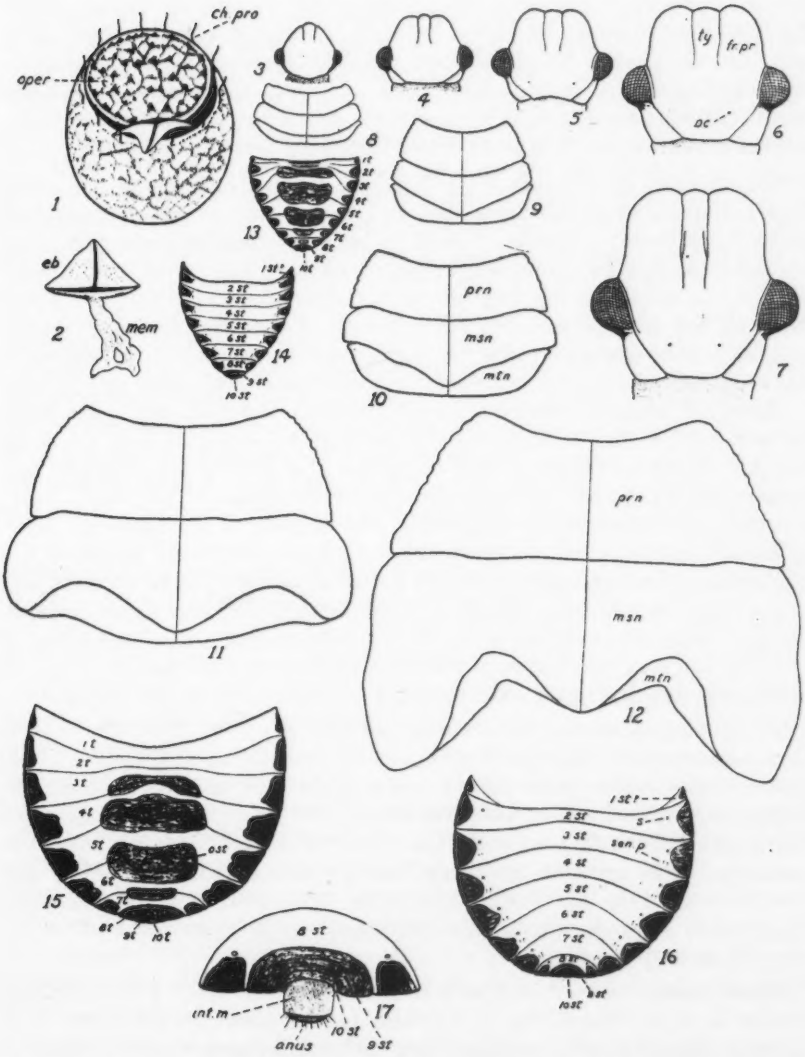
The eggs of *P. serieventris* can be readily distinguished from those of *P. maculiventris* and *P. modestus*, species of the same genus found in Cape Breton. The eggs of *maculiventris* are slightly smaller, and darker, and have a denser armature of longer spines than those of *serieventris*, and the chorionic processes are about one-third longer. The eggs of *modestus* equal in size those of *serieventris*, but are distinguished by their lighter color and smooth surface, and by the greater curvature of the chorionic processes.

The Nymphal Stages

Kirkland (7, 8) reared *P. serieventris* from the egg stage to the adult, in Massachusetts, in 1896 and 1897, and found but four nymphal stages, which he describes in detail. The writer found five nymphal stages in Cape Breton, which are described below.

First instar. Length 1.6 to 1.7 mm., width 1.05 to 1.1 mm. Form ovate, tapering more rapidly toward caudal end; convex above and below. Head dark brown, rounded on occipital margin (Fig. 3); eyes dark red, not prominent; anterior margin of tylus rounded, extending beyond frontal processes; ocelli not apparent, even in head boiled in potash. Head impunctate, sparsely clothed with microscopic yellow hairs; short, stout hairs on margin anterior to eyes; head disposed dorso-ventrally. Antennae 1.05 mm. long, four-segmented; brown, whitish at joints; segments in order of length, 1, 3, 2, 4, the latter being the longest; segment 1 stout, curved basally; segments 2 and 3 thickened distally; segment 4 widest near middle; ring joints not distinct; all segments pubescent. Beak four-segmented, stout, reaching to posterior coxae; segment 3 shortest, 1 and 2 subequal, 4 longest; apex membranous, bearing minute hairs. Labrum extends to distal margin segment 1.

Thorax (Fig. 8) dark brown dorsally, with median white sulcus; pro- and meso-nota subequal; metanotum reduced medially, widening laterally; short hairs borne on lateral margin of thorax; impunctate, but transversely furrowed. First spiracle on posterior margin proepimeron, second on posterior margin mesoepimeron. Legs brown; coxae widely separated; femora



FIGS. 1–16. *1. Hatched egg of Podisus serieventris Uhler. 2. Egg-burster, removed from the hatched egg. 3–7. Dorsal view of head capsule:—3, first instar; 4, second instar; 5, third instar; 6, fourth instar; 7, fifth instar. 8–12. Dorsal view of thorax:—8, first instar; 9, second instar; 10, third instar; 11, fourth instar; 12, fifth instar. 13. Dorsal view of abdomen of first instar. 14. Ventral view of abdomen of first instar. 15. Dorsal view of abdomen of fifth instar. 16. Ventral view of abdomen of fifth instar. 17. Caudal segments of abdomen, fifth instar, enlarged, ventral view.*

ABBREVIATIONS:—*ch. pro., chorionic process; eb, egg-burster; fr. pr., frontal process; int. m., intersegmental membrane; mem, membrane at base of egg-burster; msn, mesonotum; mtn, metanotum; oper, operculum; oc, ocellus; ost, ostiole; prn, pronotum; s, spiracle; sen. p., sensory puncture; st, sternum; t, tergum; ty, tylus (clypeus).*

widened distally, swollen near apex, anterior tibiae widest at middle, middle and hind tibiae uniform in width; on inner surface of anterior tibiae, near apex, and on anterior margin at apex, are short spurs, of consolidated setae; tarsi two-segmented, second about twice as long as first; tarsal claws light brown, curved, paired; pulvilli scoop-shaped; empodium consists of a spatulate structure bearing two long apical hairs. Sternum weakly sclerotized.

Ground color of abdomen (Fig. 13) pale yellow; on dorsum are seven brown, sclerotized medial areas; first and second narrow; third constricted medially, widening laterally; fourth to seventh roughly rectangular, the posterior two smaller, and having a medial sulcus. The paired, oval-shaped openings or ostioles of the scent glands are situated between the fourth and fifth, and the fifth and sixth, abdominal terga. Connecting the hind margins of each pair of openings is the transverse conjunctiva, which, according to Moody (10), "is a fine line marking the scar through which the gland lining is pulled out when a molt takes place." Between the third and fourth terga are two narrow slits, but these are apparently not connected with the scent glands. Along the lateral margin of dorsum is a series of brown sclerotized areas; there is one area for each segment up to the eighth; the first area is small, triangular, adjacent to the metanotum; the second to the eighth are semi-elliptical, decreasing in size posteriorly; the ninth and tenth segments are completely sclerotized. Mesad of the lateral areas are 10 to 12 bands of red, the anterior ones directed cephalo-mesally, the posterior ones caudo-mesally. On the sternum of the abdomen are lateral sclerotized areas corresponding in number, size and color to those on the dorsum; mesad of these is a row of short, irregular, transverse red bands.

There are presumably eight abdominal spiracles (Fig. 14). The first is very minute in the last three stages, and can be detected only after staining (acid fuchsin), which causes the tracheal trunk to show up as a very fine tube; it was not observed in the first three instars, but presumably it is located on the dorsum of the first segment, mesad of the lateral triangular area; the remaining seven spiracles are located on the sterna of the succeeding segments, mesad of the lateral sclerotized areas. Five pairs of sensory punctures are present, as in all other stages, behind the spiracles on the third to the seventh segments, inclusive.

Second instar. Length 2.3 to 2.9 mm., width 1.7 mm. Form elliptical, convex as in 1. Head (Fig. 4) blackish brown, more quadrate than in 1, occipital margin nearly straight; eyes red, prominent, head narrowed in front of eyes; tylus extending slightly beyond frontal processes, constricted posteriorly; frontal processes marginated laterally and anteriorly, a few microscopic hairs being borne on the margin. Tylus and frontal processes coarsely punctate, the punctures extending onto frons and vertex, but finer on these latter regions; coarse punctures bounding the mesal margin of the eye; occiput impunctate. Ocelli not present. Antennae 1.5 mm. long, segments 2 and 4 subequal; ring joints apparent at bases of segments 3 and 4; segments 2 and 3 pubescent, segment 4 with longer hairs. Basal segment

of beak black, others brown; segment 1 subcylindrical, 2 enlarged apically, 3 and 4 decreasing apically; beak flattened dorsally, convex ventrally. Labrum extends to basal portion second labial segment. In this and following instars, the head is held in more or less of a horizontal position.

Metanotum narrowed medially and laterally (Fig. 9); lateral margin of pronotum serrate, that of mesonotum slightly serrate. Thorax uniformly, coarsely punctate, somewhat rugose medially. Thoracic pleura more heavily sclerotized than in 1; sterna slightly sclerotized. Coxae closer together than in 1; tibiae broadly grooved outer surface; otherwise the legs are as in 1.

The abdomen has undergone some modification; the first medial dorsal area partly obliterated, only its lateral extremities remain; the sixth and seventh areas less distinctly divided medially. There is a more conspicuous lateral margin. The transverse red bands are longer, extending to the medial areas. On the mid-ventral region of segments 4 to 8, inclusive, are sclerotized brown areas, the first two very small; the third largest, and the last two successively smaller. The sclerotized areas on the dorsum of the abdomen are coarsely punctate; those on the sternum are impunctate.

Third instar. Length 3.8 to 4.6 mm., width 2.5 to 3.1 mm. Elliptical, widest at third or fourth abdominal segment. Head more quadrate than in II; ocelli not evident on intact head, but two minute transparent areas are visible on the moulted head capsule of this instar (Fig. 5). Antennæ 2.3 mm. long; segments in increasing order of length, 1, 3, 4, 2.

Pronotum expanded laterally; posterior margin of mesonotum extends back in V-shaped lobe, the rudiment of scutellum; on either side of medial lobe is a slight projection, the rudiment of the wing pad; metanotum partially overlapped by mesonotum (Fig. 10). Thorax rugose medially, coarsely punctate laterally and posteriorly; on each segment, on either side of the medial line, is a small smooth area. Color black, or black with two brown spots on each side of the pronotum, and a smaller spot on each side of mesonotum. Coxae near mid-ventral line; legs as in II.

Abdomen much as in II; the first medial area on the dorsum is represented only by faint lateral portions; the second is very small or obliterated, the others as in II. The red bands are more extensive, some extending completely across the dorsum.

Fourth instar. Length 5.8 to 6.7 mm., width 3.4 to 4.1 mm. Ovate, widest at third abdominal segment. Head (Fig. 6) nearly rectangular, longer than broad; ocelli distinct; antennae 3.1 to 3.4 mm. long, antennal segments as in III; beak as in III.

Caudolateral lobes of mesonotum enlarged, extending to caudal margin of metanotum; slight rounded lobes on caudolateral margins of metanotum (Fig. 11). Color of thorax variable; may be dark brown or black, with large brown areas on pro- and mesonota, and occasionally small brown spots on metanotum; or, more rarely, light brown may predominate, with dark brown

or black on mid-dorsal line and margins. First tarsal segment relatively longer than in preceding instars.

Lateral triangular area on first abdominal segment partially concealed by lobes of mesonotum; first and second medial areas almost obliterated; transverse red bands more extensive than in III.

Fifth instar. Length 7.5 to 9.0 mm., width 4 to 5 mm. Form as in IV. Tylus partially divided, posteriorly, into three parts (Fig. 7); ocelli distinct, eyes prominent. Antennae 4.3 mm. long, segment 3 relatively longer than in IV. Segments 1, 3 and 4 of beak subequal; segment 2 longest.

Lobes of mesonotum extend back onto third abdominal segment, overlying all but the mesal portions of the metanotal lobes (Fig. 12). Legs as in IV; swellings on femora less distinct.

First three lateral areas on abdomen reduced to narrow strips along the margin (Fig. 15). The eighth, ninth and tenth segments are completely sclerotized, dorsally; the ninth and tenth segments are completely sclerotized ventrally.

The coloration is variable; commonly, head and thorax are black with brown markings, antennae blackish-brown; coxae, trochanters and base of femora pale brown, remainder of legs dark brown. Ground color of abdomen cream, transverse bands red, lateral and medial areas are dark brown to black. The abdominal glands of the nymph atrophy during the fifth stage, and the metathoracic glands of the adult begin to develop at the same time; the two sets of glands, while performing the same function, are not morphologically related in any way (10).

Head Capsule Measurements

That the occurrence of five nymphal stages in Cape Breton is quite normal, is evidenced by the following data on head capsule measurements. Taylor (17) quotes Dyar (1890) as follows: "If two sets of observations show a different number of stages for the same insect but each follows its own progression, we may conclude that this variation is actual; but if either set shows a lack of regular progression that one we must regard with suspicion." The measurements shown in Table II were made on the exuviae of bugs which were reared individually after the first moult, so the instar corresponding to each cast skin was definitely known. The width of the head, between the narrow annular sclerites along the mesodorsal margin of the eye (Figs. 3-7), was found most convenient for measurement.

In calculating the theoretical head widths, the average ratio of increase was employed. The growth ratio between the first and second stages works out to 1.257, between the second and third stages to 1.323, between the third and fourth to 1.296, between the fourth and fifth to 1.230. The average of the four ratios is 1.277. The theoretical widths of the later stages were calculated by multiplying the average width of the first stage by successive powers of the ratio 1.277.

TABLE II

FREQUENCY TABLE SHOWING THE MEASURED HEAD WIDTHS OF THE NYMPHAL STAGES OF *Podisus serieventris* UHLER, IN MM.

—	1st instar		2nd instar		3rd instar		4th instar		5th instar	
	Width	No.	Width	No.	Width	No.	Width	No.	Width	No.
	.53	1	.60	1	.82	1	1.07	1	1.30	1
	.54	5	.64	2	.84	1	1.09	1	1.35	1
	.55	3	.65	1	.85	1	1.13	1	1.38	1
	.56	6	.66	5	.86	1	1.16	4	1.41	2
	.57	11	.67	1	.88	4	1.19	2	1.42	1
	.58	6	.69	1	.90	1	1.20	5	1.46	1
	.59	2	.70	1	.91	2	1.21	2	1.49	3
	.60	1	.71	5	.92	2	1.22	1	1.51	6
			.73	4	.93	3	1.24	3	1.53	1
			.74	6	.94	1	1.25	1	1.54	3
			.75	2	.95	3	1.26	3	1.56	1
			.76	2	.96	2	1.27	3	1.57	2
			.77	2	.97	1	1.29	3	1.58	1
			.78	1	.98	1	1.32	1	1.59	1
					.99	3			1.62	1
					1.00	1				
					1.02	1				
					1.03	2				
					1.06	1				
Total no.		35		34		32		31		26
Range	.53–.60		.60–.78		.82–1.06		1.07–1.32		1.30–1.62	
Arith. mean	.564		.709		.938		1.216		1.496	
Calc. mean (R = 1.277)	.564		.720		.918		1.172		1.496	
Prob. error of mean	±.00194		±.00512		±.00697		±.00715		±.01005	
Standard deviation	.0170		.0443		.0585		.0591		.076	
Coeff. variation	3.01		6.25		6.23		4.86		5.07	

TABLE III

HEAD WIDTHS OF SUCCESSIVE INSTARS OF *P. serieventris*, IN MM.

Sex of bugs	2nd instar	3rd instar	4th instar	5th instar
Males				
No. 1	.73	.88	1.16	1.46
No. 2	.60	.82	1.09	1.38
No. 3	.71	.95	1.22	1.54
No. 4	.65	.90	1.16	1.51
No. 5	.71	.93	1.21	1.49
No. 6	.73	.88	1.07	1.30
No. 7	.74	.93	1.19	1.41
Range	.60–.74	.82–.95	1.07–1.22	1.30–1.54
Average	.696	.899	1.157	1.441
Females				
No. 1	.74	.95	1.20	1.54
No. 2	.66	.86	1.13	1.49
No. 3	.67	.85	1.16	1.42
No. 4	.73	.96	1.26	1.51
No. 5	.70	.94	1.19	1.35
No. 6	.73	.93	1.24	1.51
Range	.66–.74	.85–.96	1.13–1.26	1.35–1.54
Average	.705	.915	1.197	1.470

There is some overlapping between the instars, as shown by the ranges. Considering, however, the actual means of the five instars in relation to the calculated means, it is apparent that there is a close approximation to a uniform growth ratio throughout the developmental stages. The standard deviation for each of the five instars is relatively small compared to the corresponding mean, as shown by the low coefficients of variation. The low probable error of the mean (computed by the Gaussian formula), gives evidence of a very fair degree of precision in the value of the means.

The successive head widths of 13 individuals were obtained, after the first moult. They are shown in Table III, the individuals being separated according to sex, which was determined at the final moult.

The number of individuals whose measurements are given in Table III is not as extensive as the number included in Table II, and the ranges for the instars are smaller. Because of the smaller number, the averages are less reliable than those given in Table II, although the differences are small. It will be noted that the female nymphs, on the average, are slightly larger than the male nymphs. The female adults are somewhat larger than the males, as shown previously.

Seasonal History

Emergence from Hibernation

Kirkland (7) found that all males in his rearing cages died in the autumn, without entering the soil for hibernation. He concluded that the females, fertilized in the fall, are the only sex to hibernate. Morrill (12) writes: "Pentatomids are among the earliest insects to emerge from hibernation in the spring, although apparently only a small percentage passes the hibernating period successfully. Both sexes hibernate in many, if not all, species."

The time at which *P. serieventris* emerges from hibernation, in Cape Breton, was not determined. In 1931, the first balsam fir tree was examined June 1, and a male adult was found in the foliage. Altogether only 12 overwintering adults were found, 4 males and 8 females, although balsam trees were felled onto the cotton mat, and their foliage examined, every few days. The last of the overwintering adults was collected July 29, and none survived after August 5.

Newly emerged adults sucked the juices from the new growth (the needles and the stem) of balsam fir, and the juices of *Peronea variana* larvae, in rearing vials. Although copulation was not observed in the case of overwintering adults, placed together after their capture in the field, the writer believes that copulation normally takes place during June and July. What was apparently an exceptional case of copulation occurred August 23, both the male and the female being newly matured bugs. They were observed *in coitu* at 1.30 p.m., at 4.30 p.m., at 9.30 p.m. and at 8.00 a.m. August 24, but had separated at 8.45 a.m. It is possible that the pair remained *in coitu* from 1.30 p.m. August 23 to 8.00 a.m. August 24, a period of over 18 hr.

Oviposition

Oviposition presumably follows soon after copulation. Eggs were found in the field early in July, though some must have been present late in June because first stage nymphs were found July 2. The eggs are deposited in masses, each egg resting at a slight inclination, and are cemented to each other and to the surface upon which they are deposited. They were most frequently found on the new growth of balsam fir. It seems probable that the adults are attracted by the succulent foliage, which serves also as a source of nourishment for the first stage nymphs. Eggs occurred less frequently on the old foliage of balsam, and occasionally on alder foliage. Four egg masses were found on spruce foliage, in the Gaspé balsam-spruce forest, in 1932; in this region, the most abundant insect available as food is the white spruce sawfly, *Diprion polytomum* Hartig, feeding on the spruces. It seems probable that the eggs tend to be laid near the source of food, which may be purely accidental in that the adults may lay their eggs while in search of food.

The number of eggs laid by the females was not determined exactly. Only two females laid eggs in captivity, one laying 23 in one mass, the other laying 48 in four lots of 14, 14, 14 and 6 respectively, the first lot August 28, the second September 3 or 4, the third September 8, and the final lot September 21. This female was the one observed in copulation soon after maturity, mentioned above as an exceptional case; her period of oviposition was also exceptional. Kirkland states the females lay 50 to 60 eggs.

The number of eggs to a mass ranged from 4 to 23, with an average number of 11 for 27 masses examined.

Incubation

The incubation period of the species in Massachusetts, in 1896 and 1897, was 8 to 10 days, according to Kirkland. In Cape Breton the period was somewhat longer (Table IV), and of five egg masses under observation the period ranged from 10 to 15 days.

TABLE IV

INCUBATION PERIOD OF EGGS OF *P. serieventris*

Eggs in masses	Date deposited	Date of hatching	Period, (days)	Average daily mean temperature, °F.
23	July 28	Aug. 7	10	66.6
14	Aug. 28	Sept. 11	14	61.8
14	Sept. 3 p.m. or Sept. 4 a.m.	Sept. 18	14 or 14½	60.1
14	Sept. 8	Sept. 23	15	——
6	Sept. 21	Oct. 4	13	Room temperature

The average daily mean temperatures during the periods were calculated from the daily maxima and minima, recorded in the tent laboratory.

Process of Hatching

The fully developed embryo lies within the egg shell with its head directed at right angles to the long axis of the body, and immediately under the operculum. The beak, antennae and the legs are folded upon the venter. Inside the egg shell is a peculiar T-shaped structure known as the anchor process or egg-burster which consists of a transverse bar and a median spur. A tough, transparent membrane lies at the base of the transverse bar, and between the apex of the median spur and the extremities of the transverse bar (Fig. 2). Attached to the base of the anchor process is a thinner membrane, which, according to Heidemann (6) "envelopes the young larva before it emerges from the egg." Before hatching, the anchor process partly covers the dorsum of the thorax, the median spur fitting over the mid-dorsal sulcus, and the transverse bar resting approximately along the line of union between the head and pronotum. The transverse bar thus underlies an arc of the periphery of the operculum.

When eclosion begins, the operculum is loosened all around its periphery, except for a small arc adjacent to the anterior margin of the clypeus of the embryo. This small attachment serves as a hinge on which the operculum swings upward. The operculum is apparently loosened by the upward pressure of the anchor process; Muir (see Butler, 4) considers that the egg-burster actually cuts through the chorion, around the edge of the operculum. If this is so, the transverse bar would presumably perform the cutting of the egg shell. As the nymph emerges, the operculum swings upward, and the egg-burster is drawn forward along the mid-dorsal line of the thorax and head, and then backward along the mid-ventral line. The peristaltic movements accompanying eclosion are almost imperceptible. As soon as exposed, the legs and antennae are exercised moderately. The usual sequence is as follows: the anterior legs are first exercised, then the antennae (often aided by the anterior legs) and finally the middle and posterior legs. After a short interval the legs are extended to the upper surface of the operculum, and the nymph draws itself from the egg shell. The integument of the newly emerged nymph is very soft and pale, and in the head region a continual throbbing may be observed. Usually the nymph rests on the operculum until its integument hardens and darkens.

The operculum falls back into place and the egg-burster usually protrudes through the aperture. The membrane at the base of the egg-burster is much shrunken after hatching; it is not definitely connected to the egg shell, but frequently adheres to its inner surface.

Five nymphs were timed, in the process of eclosion. The first loosening of the operculum was regarded as the starting point, and eclosion was considered complete when the nymph rested on the operculum. The observations are shown in Table V.

The percentage of hatch, based on the laboratory records and examination of field-collected egg masses, was over 90%. An unusual condition existed,

TABLE V

TIME OF ECLOSION IN *P. serieventris* UHLER

Individual	1	2	3	4	5
Time elapsing before legs and antennae were released, min.	5	6	7	7	8
Time elapsing up to resting on operculum, min.	10	9	11	12	13

in that the first nymphs to emerge from an egg mass frequently sucked remaining unhatched eggs. This is described more fully below.

Duration of the Stages

Nymphs were reared gregariously after emergence, until the first moult. In most instances, all the eggs of the same egg mass hatched within a few hours at the most; frequently, several nymphs would be emerging at the same instant. To a lesser degree, this uniformity of development among individuals from the same egg mass was also true of the successive moults.

After the first moult, most nymphs were placed in separate vials, and records on feeding and development were taken until their maturity. The individuals from two egg masses were reared collectively throughout the developmental stages, one lot in a lantern-globe cage, and the other lot in a large shell vial.

Data on the duration of the first stage are shown in Table VI. The first stadium ranged from 5 to 10 days. During the time that first-stage nymphs normally occurred in the field (July and the first half of August), the first stadium ranged from 5 to 8 days, with an average of approximately 6 days.

Data on the duration of the later stages, obtained from the individual rearings, are shown in Table VII. It will be observed that male and female bugs had practically the same duration, in the several stages.

TABLE VI

DURATION OF THE FIRST STAGE OF *P. serieventris* UHLER

Number of individuals	Date of hatching	Date of first moult	Days spent in first stage	Average daily mean temperature, °F.
13	July 8	July 13	5	72.7
5	July 9	July 14	5	72.6
6	July 13 (a.m.)	July 19 (p.m.)	6½	66.3
12	Aug. 7	Aug. 14	7	65.2
1	Aug. 7	Aug. 15	8	65.7
7	Sept. 11 *	Sept. 20	9	59.2 **
2	Sept. 11 (a.m.) *	Sept. 20 (p.m.)	9½	59.2 **
9	Sept. 18 *	Sept. 28	10	Room temperature
9	Sept. 23 *	Oct. 2	9	Room temperature

**The irregularity of eggs occurring at this time of the season has been noted under the heading "oviposition."*

***Temperature records, as well as other field records, were ended September 18, thereafter the nymphs were exposed to room temperature.*

TABLE VII

DURATION OF THE LATER STAGES OF *P. sericeventris* UHLER

Number of individual	Sex of bug*	Second stadium	Third stadium	Fourth stadium	Fifth stadium	Date bug matured
1	Male		6	8	12	Aug. 4
2	Female		6	10	13	Aug. 8
3	Male		8	6	18	Aug. 9
4	Female		8	7	14	Aug. 10
5	Female		8	8	12	Aug. 13
6	Male	7	6	8	12	Aug. 15
7	Male	8	6	9	11	Aug. 16
8	Male	8	6	8	13	Aug. 18
9	Female	11	6	8	11	Aug. 18
10	Female	8	6	10	13	Aug. 19
11	Female	7	8	8	14	Aug. 19
12	Female	7½	5	9	15	Aug. 20
13	Female	8½	6	9	18	Aug. 25
14	Male	6	8	8	14	Aug. 26
15	Female	9	6	8	25	Sept. 1
16	Female	7	9	10	16	Sept. 1
17 **	Female	7	8	8½	17	Sept. 23
18 **	Male	6	9	10	19	Sept. 27
19 **	?	8	10	9½	19½	Sept. 30
20 **	Male	10	9½	11½	18½	Oct. 2
Male bugs {Range		6–10	6–9½	6–11½	11–19	
Male bugs {Average		7½	7½	8½	14½	
Female bugs {Range		7–11	5–9	7–10	11–25	
Female bugs {Average		8	7	8⅔	15¼	

**The sex of individuals was determined after the final moult.*

***Individuals Nos. 17 to 20, inclusive, were fed on animal food alone; all others were provided both animal and vegetable food.*

The data obtained from the two collective rearings are summarized in Table IX. It will be observed that the earlier moults were very close in point of time, among individuals from the same egg mass. As development continued, the cumulative effect of small differences among individuals became more pronounced; in lot B, there was a spread of 11 days between the earliest and latest moult into adult stage.

The total developmental period, from hatching until the final moult, was determined for 23 individuals. The data are shown in Table X, and relate to those nymphs (whose more detailed histories are included in Tables VII and IX) which were under observation throughout the whole developmental period. The developmental period ranged from 37 to 55 days, when hatching occurred in early July, and from 47 to 58 days, when hatching occurred in early August. The average period of the 23 individuals was 45 days. Very nearly the same average period is obtained by totaling the average duration of the five stadia, as shown in Table VIII.

TABLE VIII

AVERAGE DURATION OF THE FIVE STADIA IN *P. serieventris* UHLER

						Total
Stadium	1	2*	3*	4*	5*	
Male, days	6	7½	7⅓	8½	14⅔	44
Female, days	6	8	7	8⅔	15¼	45 (approx.)

*Table VII.

TABLE IX

SUMMARY OF LIFE HISTORY OF TWO LOTS OF NYMPHS REARED COLLECTIVELY

Lot	1st moult		2nd moult		3rd moult		4th moult		5th moult	
	Date	No.	Date	No.	Date	No.	Date	No.	Date	No.
A.....6 eggs										
Hatched July 12	July 19	6	July 24	6	July 30	5*	Aug. 7	4	Aug. 19	1
							Aug. 8	1	Aug. 20	4
B.....5 eggs	Aug. 14	4	Aug. 21	4	Aug. 29	2	Sept. 7	1	Sept. 23	1
Hatched Aug. 7	Aug. 15	1	Aug. 23	1	Aug. 30	2	Sept. 8	2	Sept. 24	1
					Sept. 7	1	Sept. 10	1	Sept. 26	1
							Sept. 19	1	Sept. 29	1
									Oct. 4	1

**One of the third-stage nymphs escaped July 28.*

TABLE X

TOTAL DEVELOPMENTAL PERIOD, *P. serieventris* UHLER, IN DAYS

Date of hatching	Date of final moult	Total developmental period	Sex of bug	Date of hatching	Date of final moult	Total developmental period	Sex of bug
July 8	Aug. 15	38	Male	July 13	Aug. 20	38	Female
July 8	Aug. 16	39	Male	July 13	Aug. 20	38	Female
July 8	Aug. 18	41	Female	Aug. 7	Sept. 23	47	Female
July 8	Aug. 19	42	Female	Aug. 7	Sept. 23	47	Female*
July 8	Aug. 19	42	Female	Aug. 7	Sept. 24	48	Female
July 8	Sept. 1	55	Female	Aug. 7	Sept. 26	50	Female
July 9	Aug. 18	40	Male	Aug. 7	Sept. 27	51	Male*
July 9	Aug. 20	42	Female	Aug. 7	Sept. 29	53	Female
July 9	Aug. 25	47	Female	Aug. 7	Sept. 30	54	? *
July 13	Aug. 19	37	Male	Aug. 7	Oct. 2	56	Male*
July 13	Aug. 20	38	Male	Aug. 7	Oct. 4	58	Female
July 13	Aug. 20	38	Female				

Range: 37–58 days Average, 45 days

**These individuals were fed on an animal diet alone, during the last four stages. All others were provided with both animal and vegetable food.*

Process of Moulting

Feeding generally ceases two or more days before moulting occurs. This cessation is necessary, because the chitinous parts of the salivary and pharyngeal pumps are cast out and replaced at ecdysis. Before ecdysis, the white areas turn dull gray, the integument is tightly stretched, and motions are jerky and uncertain.

The nymph prepares for moulting by spreading its legs widely, thrusting its head forward, the labium beneath the thorax, and the antennae beneath the thorax or to the sides. The head capsule splits along the paracephalic sutures, and the corneas of the compound eyes separate from the head capsule on the dorsal, anterior and anteroventral margins. The thorax splits along the mid-dorsal sulcus, and the pronotum is drawn away from the mesonotum. In the moulting of the fifth instar, the wing pads are elevated and the mid-dorsal split extends to the apex of the scutellum.

When ecdysis begins, the lateral halves of the thoracic terga are forced apart, exposing the thorax of the succeeding instar. The thorax is considerably humped, and the shortening of the body, accomplished by this humping, enables the head to be withdrawn from the old capsule. The thoracic segments are released by a series of peristaltic movements. The labium and antennae are drawn from their old coverings by the same movements that effect the release of the thorax and abdomen. The mandibular and maxillary setae project beyond the tip of the labium, both pairs curving lateroventrally, the former more strongly than the latter. As the setae are withdrawn a white sheath is slid from each, a phenomenon which is apparently the same as that which Snodgrass (15) found during moulting of the cicada. The basal segments of the legs are soon exposed, and the soft integument near the femorotibial joint alternately collapses and resumes the normal contour, a process which apparently assists in the release of the tarsal segments. The legs are then put through a series of moderate exercises, thus gradually gaining strength. The old chitinous linings of the tracheae are pulled through the spiracles of the new instar; they are readily observed being drawn through the thoracic spiracles, appearing as long, tapering white tubes. The abdomen is withdrawn from the collapsing skin by a series of bending movements, and frequently the hind tibiae are employed to thrust back the exuviae.

When the fifth instar moults, the delicate wings are exposed. The scutellum, horizontal at first, soon curves up apically, possibly to ensure more rapid hardening of the wings. The hind wings and the membranous portion of the fore wings are white, wrinkled and sac-like, becoming lamellate in a few minutes; this change may be assisted by a patting and stroking action of the posterior tibiae. The scutellum soon drops back into position. The genital segment of male adults protrudes at first, but is soon telescoped within the preceding segments. Two fifth stage nymphs, moulting into the adult, required 14 and 23 min. respectively, from the first splitting of the thoracic terga to the complete shedding of the exuviae.

Number of Generations

It has been pointed out that overwintering adults may be found in the trees during June, July and the first few days in August. Adults of the current generation appear quite early in August. In 1930, the first adults occurred in the field laboratory August 6; in 1931 the first adult in the laboratory rearings appeared August 4, and the first field-collected adult

of the current generation was taken August 14. Newly matured adults are recognized by their fresh appearance, and the entirety of the wing membranes; the wing membranes of overwintering adults are usually quite tattered. Most individuals of the *Podisus* population were in the adult stage by the end of August, though a few retarded individuals matured during September, and even in October (indoors).

Early maturing adults did not generally copulate in the late summer. Presumably this function takes place after the emergence of the two sexes in the spring. The one female that was known to be fertilized shortly after her maturity, in August, laid her eggs in August and September. Due to the lack of insect food, and to the low temperatures, there is little likelihood that the ensuing nymphs, which emerged during September and October in glass vials, would have been able to survive under field conditions. It seems improbable that there was more than one complete generation, in Cape Breton, in 1930 and 1931.

Observations in the Berry Mountain Brook district of central Gaspé indicate a similar life history of *P. serieventris* in that region, to that in Cape Breton. Overwintering adults, including one male, were collected early in July and again in the third week of August, 1932. Field-collected eggs hatched July 12 and September 9. Development was slow, as indicated by observations on a few individuals in vials, kindly supplied by Mr. W. A. Reeks. The duration of the first stage was 11 days; of the second, 15 days; of the third, 17 days; and of the fourth, 11 days. The individuals died before the fifth moult. Fifth-stage nymphs appeared early in September in the field; one of these moulted into the adult stage early in October, in the laboratory. The eggs which hatched September 9 were apparently deposited by one of the longer-lived overwintering females, as there was insufficient time for a generation to have matured and deposited eggs by the first of September. In all probability there was but one complete generation in central Gaspé, in 1932.

In Massachusetts, Kirkland reared two generations of *P. serieventris* between the last of June and the last of September; he believed that the progeny of the overwintering adults had matured by the last of June, and concluded that there were three generations a year in that region.

Hibernation

The time at which the adults enter into hibernation was not determined. Adults were seen in the field, in October 1930, at St. Peters. Field work at Grand River in 1931 ceased September 18, and at that time adults were still in the trees. In the absence of direct observation the writer presumes that the adults burrow into the forest litter in Cape Breton, as stated by Kirkland to be the case in Massachusetts. Both sexes hibernate in Cape Breton.

Sex Ratio

The sex ratio, based on laboratory rearings and collections in the field in September 1931, before hibernation commenced, was between .55 and .65. The number of adults secured was not extensive enough to justify a more exact statement of the ratio.

Value as a Predator

Habits of First Stage Nymphs

The first stage nymphs are definitely gregarious. For the first day or so after emerging, they remain huddled on the egg mass, those on the outside with their heads directed inwards. Nymphs in rearing vials ranged out over the foliage a day or two after emerging, and sucked the juices from the new foliage of balsam fir and white spruce, and from the leaves of alder and lilac. Almost any part of the leaf was punctured; in a few cases, the setae were applied to the stomata of balsam needles, but this may have been without significance. Newly emerged nymphs were occasionally found sucking from unhatched eggs of the egg mass from which they originated. Morrill (12) states that first-stage nymphs of *Pentatoma ligata* Say have the same habit, and examination by the writer of several egg masses of *Podisus modestus* indicated a similar habit for this species. The operculum of sucked eggs is not loosened, and the shell almost invariably collapses after the contents is withdrawn. Of 203 *P. serieventris* eggs collected at Grand River in 1931, 19 had been sucked; of 48 eggs collected on white spruce in Gaspé in 1932, 5 had been sucked. About half of the collapsed eggs were practically empty, one-quarter contained a dried yellow substance, and the remainder contained dried irregular bodies in which a suggestion of segmentation was detected. Apparently most of the collapsed eggs had undergone little or no development at the time they were sucked, and judging by the simultaneity which characterizes hatching in the same egg mass, it seems probable that these eggs were infertile. There had been some development in about one-quarter of the collapsed eggs, evidenced by the dried segmental contents, and in these instances the young nymphs may actually have killed the embryos. It is of course possible that the embryos had previously died, and the young nymphs merely withdrew the unused food contents of the eggs.

There has been considerable discussion relative to the feeding habits of the first-stage nymphs of species of *Podisus*. Whitmarsh (23) writes of *P. maculiventris* as follows: "These insects are entirely predaceous, except possibly to a slight extent during the first instar when they may suck plant juices if they feed at all, which thus far is unproved." Stoner (16) describes the habits of first-stage nymphs of the same species, in the presence of celery leaf-tyer caterpillars; the nymphs did not feed on the caterpillars for 15 days after hatching, though some appeared to feed on celery sprigs, and showed cannibalistic tendencies as well. Kirkland (7) states that the first stage nymphs of *P. serieventris* were fairly active five days after hatching, without once having had food. He also found that the nymphs fed on eggs of the

gipsy moth, but died if given no other food, and concluded that their death was due to insufficient nourishment.

Concerning several species of pentatomids studied in relation to the Mexican bean beetle, Plummer and Landis (14) write as follows: "First-stage nymphs of all species of pentatomids observed failed to feed on larvae of *E. corrupta*. As soon as the first molt occurred the nymphs attacked the larvae."

The writer placed small *Peronea variana* caterpillars in rearing vials with first-stage nymphs of *Podisus serieventris*, but in no instance did the latter feed upon the caterpillars. Experiments were carried out to determine (1) whether nymphs could pass the first stage without feeding at all; (2) whether they could subsist on vegetable food alone; (3) what stage of their development they could attain when the vegetable diet was continued.

(1) Thirteen nymphs were placed in vials without food. They were examined daily, and dead nymphs removed to prevent others from feeding upon their juices. All nymphs were feeble after the third to the fifth day. Two individuals lived for 4 days, 1 nymph for 5 days, 4 nymphs for 6 days, 2 nymphs for 7 days and 4 nymphs for 9 days. None, however, were able to survive till the first moult.

(2) In the ordinary rearing experiments, the majority of the nymphs survived the first stage when foliage was supplied. As a special check on the rate of survival, 40 newly emerged nymphs were placed in vials in lots of 14, 13, 9 and 4 respectively. Balsam, white spruce, alder, lilac or cherry foliage was supplied, and replenished every two or three days. Dead nymphs were removed as soon as found. Nine nymphs died in the first stage, 6 to 10 days after hatching occurred. Thirty-one nymphs (about 78%) moulted the first time, 7 to 10 days after hatching; the average duration of the first stage was 9 days.

(3) The 31 survivors, then in the second stage, were supplied with foliage only. Not one lived to moult the second time, but all died from 1 to 15 days after the first moult; the average length of life in the second stage was about 6 days. The total failure of these nymphs to survive until the second moult cannot be attributed to unsatisfactory conditions in the vials, because in the rearing experiments where animal food was provided as well as vegetable food, the normal mortality at this time of life was not over 10%. The death of these individuals was apparently due to the inadequacy of vegetable food, during the second stage.

Feeding of Later Nymphal Stages

Kirkland (7) found that at the various stages *P. serieventris* consumed larvae at the following rates, on the average: first instar, 4 larvae; second instar, 6 larvae; third instar, 4 larvae; fourth instar, 11 larvae; adult, 5 larvae. In the 30 days between hatching and hibernation, 30 larvae were killed, an average of one a day.

Preliminary feeding experiments, in 1930, indicated that the bugs killed one *Peronea variana* larva every three days, as an average rate. More

extensive records were obtained in 1931, and the following insects were used as host forms: *Peronea variana* (all larval stages), *Ellopia fiscellaria*, *Hyphantria cunea*, *Galerucella alni*, and a spittle bug (*Clastoptera* sp.) from alder. Twenty nymphs were reared individually, after the first moult, on both animal and vegetable food; 12 of these nymphs finally moulted into the adult form. Four nymphs were reared individually to the adult stage on animal food alone, and two lots of bugs were reared collectively from the egg to the adult stage on an animal and vegetable diet.

Without giving the detailed records, Table XI summarizes the more important observations on the food consumption of the last four nymphal stages, based on the individual rearings where animal and vegetable food was provided.

TABLE XI

SUMMARY OF DATA ON FOOD CONSUMPTION OF LAST FOUR NYMPHAL STAGES OF *P. serieventris* UHLER

	2nd instar	3rd instar	4th instar	5th instar
Maximum number of larvae consumed by one individual	3	4	8	11
Minimum number of larvae consumed by one individual	1	1	2	3
Average number consumed	2⅓	3¼	4¼	6⅛

The greatest number of larvae consumed by one nymph during the last four instars was 19; the least number was 12; and the average number was 15. This is equivalent to a little more than one larva per nymph, every three days, between the first and the final moult.

The nymphs reared collectively consumed on the average a smaller number of larvae than those reared individually. It may be that the habit of co-operation among individuals, during feeding, eliminated waste of food and enabled the nymphs to mature on a smaller number of larvae, than was possible in the individual rearings. Table XII summarizes the observations on food consumption, in the collective rearings.

TABLE XII

SUMMARY OF FOOD CONSUMPTION IN THE COLLECTIVE REARINGS

	Lot number	2nd instar	3rd instar	4th instar	5th instar	Total, 4 instars
Average number of larvae consumed by one individual	A	1½	1	4	4¼	10¾
	B	1⅓	3⅓	2⅔	4	11

Four nymphs reared solely on animal food consumed a greater number of larvae than those provided with both animal and vegetable food. In these experiments, fall webworm larvae were used, and it was found advisable to disable them by piercing the thorax with a dissecting needle, or by crushing the thorax with forceps, so that they could not hopelessly entangle the *Podisus* nymphs in their webs. The greater food consumption of these nymphs may be due to the greater ease with which they obtained their food. The survival of these nymphs proved their independence of vegetable food, during the last four instars, provided enough insect food is available. The data obtained in these rearings are summarized in Table XIII.

TABLE XIII

NUMBER OF DISABLED FALL WEBWORM LARVAE CONSUMED BY NYMPHS OF *P. serieventris*

	2nd instar	3rd instar	4th instar	5th instar
Maximum number of larvae consumed by one individual	8	14	11	9
Minimum number of larvae consumed by one individual	5	5	5	3
Average number consumed	6	9¼	7¼	5¾

The greatest number of larvae consumed by one nymph during the last four instars was 34 larvae; the least number was 21; and the average number was 28¼.

Dependence of Nymphs on Animal Food

A few experiments were carried out to discover whether nymphs, having fed on animal food during the early instars, could thereafter subsist on a vegetable diet alone. The nymphs were captured in the field at various times and provided both animal and vegetable food until the succeeding moult. After the moult, the nymphs were supplied with vegetable food only (alder, balsam, cherry or lilac). Only a limited number of nymphs were used, but in all cases death occurred before the final moult. In all but two instances, death occurred in the same instar in which the vegetable diet was begun. The two exceptions were nymphs which completed the fourth stage on a purely vegetable diet, but succumbed in the fifth stage. The number of days upon the vegetable diet ranged from 8 to 38, with an average of 18 days, before death eventually occurred. The normal mortality of the later stages in rearing vials was approximately 35%, so the total mortality of the experimental nymphs on a vegetable diet can hardly be attributed to an unfavorable environment. It appears that *Podisus serieventris* nymphs of the later stages require animal food in order to develop to maturity.

Feeding of New Adults

Twenty-six adults were kept under observation from the final moult until their death. Of these, ten males and ten females were paired, each pair being placed in a separate vial, and six adults were placed individually in vials. Foliage and fall webworm larvae were supplied, the larvae being replaced as devoured, and the foliage renewed every few days. New adults were frequently observed sucking the juices from foliage, with living larvae untouched in the same vial.

In most cases, the adults did not begin to feed until a few days after the final moult. After feeding began, it was continued fairly regularly until the middle of September; after that, very little feeding was done. Field work ceased September 18, and thereafter the adults were kept indoors, the last individuals dying October 3. The total number of larvae consumed by the adults reared individually are shown in Table XIV.

TABLE XIV

TOTAL NUMBER OF LARVAE CONSUMED BY ADULTS OF *P. serieventris* REARED INDIVIDUALLY

Experimental number	C	D	E	F	G	H
Sex	Female	Male	Male	Female	Female	Female
Total number of larvae consumed	8	24	13	13	16	7

NOTE:—*Average number for the six adults, 13½.*

The food consumption of the adults reared in pairs was relatively less than that of the adults reared individually. This may have been due to the co-operation among individuals, as was suggested for the nymphs. The total numbers of larvae consumed by the pairs of adults are summarized in Table XV.

TABLE XV

TOTAL NUMBER OF LARVAE CONSUMED BY PAIRS OF ADULTS OF *P. serieventris*

Experimental number	I	J	K	L	M	N	O	P	Q	R
Total number of larvae consumed	48	26	16	25	19	24	24	19	15	10
Average number of larvae per adult	24	13	8	12½	9½	12	12	9½	7½	5

NOTE:—*Average number, for the 20 adults, 11½.*

The results of a small series of experiments indicated the dependence of adults on animal food; plant food was apparently insufficient to sustain life for long. The average adult life of 5 individuals deprived of all food after the final

moult was 15 days. The average adult life of 3 individuals supplied plant food was 23 days. And the average adult life of the 26 individuals supplied both animal and vegetable food was over 63 days.

Process of Attack and Feeding

That *Podisus serieventris* is a capable predator is evidenced by the following observations. A second-stage nymph was found attached by its mouth parts to the genitalia of an adult cercopid, *Aphrophora parallela* (Say), in flight at the time of capture. Adult bugs were observed feeding on the moths of *Peronea variana* and *Ellopia fiscellaria*, and on an adult plecopteron. Very small nymphs can overcome caterpillars many times their size.

The younger nymphs (second and third instars) are usually more active in their search and more aggressive in their attack, than are the older nymphs and adults, which usually capture their prey by stealth. Cleansing of the beak and antennae was frequently observed just prior to an attack, though the same operations were occasionally performed independently of an attack. In the process, the beak is extended, and the anterior legs are raised to grip the apex, one on each side; a transparent liquid may be ejected from the apex of the beak, and bathed over the tarsal segments and the distal portions of the tibiae. The tibiae are pressed against the sides of the beak, and the legs straightened; the tibiae are thus pressed along the beak apically. This may be repeated several times. The apical segments of the antennae are cleaned in a similar manner. Loosening of the setae in the labial groove is corrected by forcing the spurs of the anterior tibiae along the groove, distally.

In the approach to a victim, the extended antennae commonly are in a state of rapid vibration. The beak is held tip down, until close; here the bug usually stops, sets its legs, and makes contact with its victim by "leaning" forward. There is no definite selection of a soft area for the insertion of the setae; any part of the body wall, a proleg, the clypeus, the vertex or other sclerotized parts are successfully pierced. After the setae are inserted, there usually follows a terrific struggle, the victim thrashing about, often attempting to crush the beak with its mandibles. In most cases, attempts to gain release are futile.

The means by which the bug retains its grip has been questioned. The beak itself has no gripping function, but merely holds the setae together. The mandibular setae are rather coarsely barbed, and are forced into the tissues to a depth equal to about one-third the last labial segment. The maxillary setae are sharply pointed, without barbs; they are not forced deep into the tissues until the actual feeding begins. It has been the general opinion that the mandibular setae secure the prey. Baker (1) discredits this explanation and describes a reciprocating action of the setae, which have a strong tendency to curl inwards and backwards when released from the restraining pressure of the opposite seta of the pair, and suggests that the curling of the extended setae serves to secure the victim.

The prey becomes feeble in 3-15 min. after the setae are inserted, and the point of insertion is usually swollen and discolored. When the struggling

is over, the maxillary setae are thrust deep into the body; this is accomplished by the complete flexion of the first and second labial segments. The rapid probing movements of the setae are readily observed in *Peronea variana*, which is light green in color, with few hairs. There are occasional short periods of rest, possibly to allow the withdrawal of the larval juices. When one part of the body is emptied, the integument collapses, and the adjacent intact tissues are drawn within reach of the setae, to be broken down and pumped out. A peculiar pumping action, marked by alternate lifting and lowering of the antennae, is characteristic of feeding on animal food; this is less noticeable when the bugs feed on plant juices.

Second-stage *Peronea* larvae were sucked in 10 min., fully grown *Peronea* larvae in two to three hours. Nymphs, and adults as well, frequently co-operate in sucking from the same victim, in rearing cages.

In sucking plant juices the beak is applied more or less perpendicularly to the plant surface, and the setae extend only a short distance into the tissues. When feeding from deciduous foliage, the setae are frequently inserted in the larger veins on the lower surface.

Poisoning

Predacious pentatomids have been suspected of utilizing a poisonous secretion by numerous investigators, including Muir and Kershaw (13), McDermott (9), Baker (1), and Tothill, Taylor and Paine (18).

Several observations suggested the presence of such a secretion in *Podisus serieventris*. (1) Small globules of a transparent liquid were observed on the tips of the beaks, after unsuccessful attacks upon caterpillars. (2) Caterpillars were frequently found dead in the rearing vials, without having lost their body juices. (3) Victims usually ceased their struggles soon after being pierced, before *Podisus* started to withdraw their juices. (4) Swelling and discoloration usually marked the point of insertion of the setae.

To test the possible occurrence of a toxic substance in *Podisus serieventris*, a number of larvae of different species were introduced into vials containing nymphs and adults. The bugs were allowed to attack the larvae and maintain their grip for a short period; they were then disengaged by means of a pencil or forceps. The larvae were isolated in vials with foliage, and their subsequent behavior noted.

Eighteen larvae were exposed to attack, for 3-90 sec. Twelve larvae died about an hour, or longer, after the attack. The six larvae that survived suffered some discomfiture, evidenced by feebleness and cessation of feeding. Death of the 12 larvae was not due to mechanical injury, because larvae pierced through the thorax with a dissecting needle resumed feeding after a day, and survived. This form of injury was much more severe than the mechanical injury caused by the setae of *Podisus*.

It seems probable, therefore, that *Podisus serieventris* is supplied with a toxic substance, presumably a secretion of the salivary glands, which nearly always causes local irritation, very often causes general feebleness, and in a

considerable number of cases causes death. Its effects may be correlated with the duration of the attack, but this was not determined.

Host References

Insects preyed upon by *Podisus serieventris* are enumerated below; the list includes species referred to in the literature, and species which came under the writer's observation. To avoid unnecessary detail, the authorities for the various host species are not included, and the species names are transcribed as they appear in the literature. Species which were killed by *P. serieventris* in confinement are followed by the letter *c*, and those species which came under the writer's observation are followed by the letter *a*. All of the latter refer to Cape Breton, with the exception of *Diprion polytomum* Hartig, the record of which refers to Gaspé, Quebec, 1931 and 1932; and *Fenusa pumila* Klug, which refers to Fredericton, N.B., 1932, reported by Mr. C. C. Smith.

Lepidoptera.

Gelechiidae : *Recurvaria* sp. (probably *gibsonella*) (*a*) (*c*).
Tortricidae : *Peronea variana* (*a*).
Geometridae : *Ellopia fiscellaria* (*a*); *Nematocampa limbata* (*a*) (*c*); *Paleacrita vernata*.
Notodontidae : *Datana ministra* (*c*).
Lymantriidae : *Euproctis chrysorrhea*; *Notolophus antiqua*; *Orgyia leucostigma; Porthetria dispar*.
Noctuidae : *Noctua c-nigrum* (*c*); *Pyrophila pyramidoides*. *Rhynchagrotis alternata*.
Arctiidae : *Hyphantria cunea*.
Citheroniidae : *Anisota senatoria* (*c*); *Dryocampa rubicunda* (*c*).
Saturniidae : *Attacus cecropia* (*c*); *Attacus promethea; Telea polyphemus* (*c*).
Lasiocampidae : *Clisiocampa americana; Clisiocampa disstria; Tolype velleda*.
Nymphalidae : *Euvanessa antiopa; Limenitis ursula*.

Coleoptera.

Coccinellidae : *Adalia bipunctata*.

Hemiptera.

Pentatomidae : *Menecles insertus; Podisus cynicus; Podisus serieventris* (cannibalistic).

Homoptera.

Cercopidae : *Clastoptera* sp. (*a*) (*c*). *Aphrophora parallela* (*a*).

Hymenoptera.

Cimbicidae : *Cimbex americana* (*c*).
Tenthredinidae: *Diprion polytomum* (*a*). *Fenusa pumila*.

In a recent paper, Plummer and Landis (14) reduced the number of *Epilachna corrupta* larvae, pupae and adults, consumed by various predators,

to an arbitrary basis. They state: "It was not always possible to supply a large number of individuals of this host of the same size and stage. For this reason it would be impossible to collate the value of the several species of predators without reducing the quantity of food consumed to some standard measure." An arbitrary value of unity was assigned to a third instar larva, and the other stages were evaluated in proportion to their relative weight. "By means of the values assigned above, 'food equivalents' have been determined from the actual laboratory data. A food equivalent may be defined as the quantity of food consumed by one individual in one day." This seems to be a useful method of accurately evaluating the food consumption of different species, of predators; but as an expression of the "value" of a predator, it seems to neglect the fact that it is not the weight of the insects, but the number of insects, destroyed, that is of real significance. The destruction of ten small larvae is certainly more significant, in terms of control, than the destruction of one full grown larva ten times the weight of the smaller ones. The distinction is submerged in reducing food consumption into "food equivalents."

Relation Between Podisus Serieventris Uhler and Peronea Variana Fernald, in Cape Breton*

Reference to the epidemic of *Peronea variana* in Cape Breton has already been made in the introduction of this paper. It is not possible here to enter into a complete discussion of the biology of *Peronea*, but the more important points in its life history are outlined below.

Life History of Peronea variana

Peronea variana overwinters in the egg stage, the eggs being deposited during September on the needles of balsam fir, white spruce and to a less extent on red and black spruce. Hatching occurs during the last few days in May and the first few days in June. There are five larval stages. The young first-stage larvae burrow through the bud scales or crawl into the newly expanded shoots. Here the larvae feed during the first three stages, constructing small shelters of silk about themselves. As the new needles of balsam fir expand into a horizontal plane, and as defoliation progresses, there is a modification in the feeding habit, which generally affects the fourth- and fifth-stage larvae. Tubular, silken shelters are constructed along the stems, at the base of the new needles, which are devoured by the sheltered larvae. If defoliation is so severe that the new foliage is exhausted, the larvae move back and subsist on the old foliage.

Prior to pupation, which occurs from late in July to early in August, a more compact shelter is constructed among the excrement and dead needles; here the pupa transforms into the moth. The pupal stage ranges from 18 to 23 days. Moths are in flight during August and September, and oviposition is about finished by the end of September.

* *All data relating to the life history and population of Peronea variana Fernald have been obtained from the as yet unpublished 1930 and 1931 reports of the Division of Forest Insects, Dominion Entomological Branch.*

Stages Susceptible to Podisus Attack

Kirkland (7) records the sucking of gipsy moth eggs by the first stage nymphs of *Podisus serieventris*. Felt (5) states that *Podisus placidus* sucks the eggs of *Anisota senatoria*. It was not determined whether the eggs of *Peronea variana*, present during eight to nine months of the year, are actually sucked by *Podisus*. Numbers of perfectly formed, but empty and transparent egg shells were found in the spring of 1930; many of these showed no evidence of an emergence hole. Newly deposited *Peronea* eggs were placed in vials with adult bugs, but none were sucked after several days in the vials. It is therefore uncertain whether the empty transparent shells, found in the field, had been sucked by the bugs.

The first three larval instars are well protected in the compact new shoots. The fourth and fifth instars are more susceptible, and the pupal stage, with its long period of inactivity, is most open to attack. The adult moths are occasionally killed by *Podisus*, but the numbers thus destroyed are probably insignificant.

Podisus as a Factor in the Outbreak

Examination of the area infested by *Peronea variana*, in August 1929, showed the outbreak to be widespread and the larvae to be fairly abundant. A high percentage of hatch was recorded in June 1930, and there was apparently low mortality among the early stage larvae. *Podisus* nymphs were found in fair numbers late in July, and the adults appeared early in August. The bugs took their toll of the budworms, but no estimate of the proportion of the larval population destroyed by *Podisus* in 1930 is possible. The larval population suffered a great reduction during the final stage, due to the operation of control factors other than *Podisus;* the number surviving to the pupal stage was about 200 for the average mature balsam tree in the area of medium infestation, and approximately 2000 for the average mature balsam in the heavily infested area. The activity of *Podisus* during the pupal stage of its host was evidenced by the occurrence of sucked pupae; these were devoid of contents, with the pupal skin intact. Results with four sample collections of pupae, relative to the control of the pupal stage by *Podisus* are shown in Table XVI.

TABLE XVI

PERCENTAGE OF BUDWORM LARVAE DESTROYED BY *P. serieventris*

Sample number	Locality	Number of pupae in sample	Percentage sucked by *Podisus*
1	St. Peters	301	14
2	Grand River	493	8 (approx.)
3	Grand River	1028	5
4	Louisburg	1263	8

In addition to empty intact pupal skins, another type was frequently found, containing the dead adult, or pre-imago. These may have been sucked by *Podisus*, or may have died from other cause. Because of this uncertainty, dead pupae of this kind were not included in the percentage shown above.

There was a heavy flight of moths in September 1930, but comparatively few eggs were laid. The egg population overwintering from 1930 to 1931 was at the approximate rate of 4000 to 6000 for the average mature balsam tree in the heavily infested area, only about one-tenth the egg population of the previous year in that area.

The eggs hatched late in May, and the first few days of June, 1931. The *Peronea* population suffered great reduction between the first of June and the tenth of July, when the young nymphs of the current generation of *Podisus* began to appear in numbers. The nymphs were most numerous the latter part of July, the population ranging from 14 to 35 individuals per mature balsam tree. The budworm population was at this time greatly depleted, due chiefly to parasites and a disease which was very prevalent. The flaccid bodies of dead budworms, whose death was attributed to predatism by *Podisus*, comprised as much as 30% of the *Peronea* population in one count made early in August; in other counts, few of the sucked remains were found. The difficulty of finding the dead bodies, many of which must have dropped from the trees, and of distinguishing between parasitized and sucked remains, made estimation of the control afforded by *Podisus* a matter of uncertainty. Dead flaccid budworms, however, comprised less than 10% of the larval population found on 12 trees, examined between July 10 and August 11.

The budworm population was almost wiped out by the operation of natural control factors, before the period of pupation arrived. Accordingly, the activity of *Podisus* at this time could not be evaluated in terms of sucked pupae. The *Peronea* outbreak was so reduced that the writer did not see a single moth in the balsam-spruce forests about Grand River, in 1931.

At the end of July, the *Podisus* population ranged from 14 to 35 individuals per mature balsam tree. Of 17 trees examined during August, the number of bugs per tree ranged between one and ten; and of four trees examined early in September, the greatest number of bugs per tree was four. Rearing experiments, already described, indicated the inability of *Podisus serieventris* to subsist for long on a purely vegetable diet. Presumably the heavy reduction in the *Peronea* population during the season caused a corresponding reduction in the *Podisus* population through starvation, for no other factors of natural control were found operating against *Podisus* in the Grand River forest.

The conclusions drawn from this study agree in essentials with those reached by Plummer and Landis (14), who, in regard to the relation between predacious insects and *Epilachna corrupta* Muls. in Mexico, state: "The peak of the larval population of the bean beetle is reached and damage to bean foliage accomplished before an appreciable number of pentatomids appear in the fields. As the winter survival of *E. corrupta* in most parts of the infested areas of Mexico is high, it is likely that the number of *E. corrupta* destroyed by predators during the late summer does not greatly reduce the number of beetles necessary to maintain the bean beetle the following season." The writer's observations on *P. serieventris* are also in agreement with the

general conclusion reached by Weiss (22); of predacious pentatomids, Weiss states they are "important in a limited way..... as a whole they lack elasticity, and do not, on account of their limited powers of reproduction, respond to any sudden increase in phytophagous forms. Beyond a certain point it is useless to expect more from them....." It is very probable that the *Podisus* population increased during 1929 and 1930 when *Peronea* was present in large numbers; but in 1931, the greatly reduced host population limited in a very definite manner the number of predators that were able to reach maturity. The value of *P. serieventris* as a predator of forest insects would be greatly increased if it were capable of maintaining its population, during the decline of its host, by subsisting on vegetable food.

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STERILIZATION OF NARCISSUS BULBS BY IMMERSION IN SILVER NITRATE — POTASSIUM CYANIDE SOLUTION IN VACUO[1]

By W. Newton[2], R. J. Hastings[3] and J. E. Bosher[3]

Abstract

Through the use of a dye solution, evidence was obtained that a liquid disinfectant may be forced into the narcissus bulb parts invaded by nematodes and fly larvae by immersion *in vacuo*.

An investigation of the lethal properties of solutions against nematodes and their influence upon bulb growth led to the selection of a silver nitrate solution as a promising disinfectant, but owing to instability of silver nitrate in the presence of chlorides and other substances in tap water and in dirt clinging to bulbs, its use had no commercial possibilities. However when silver salt was combined with potassium cyanide in the ratio of 1 to 3 by weight, an effective solution of satisfactory stability was obtained.

A solution of silver nitrate 0.05% and potassium cyanide 0.15% by weight, forced into narcissus bulbs by an evacuation process, effectively destroyed bulb nematodes and bulb fly larvae without significant injury to bulb growth under greenhouse conditions.

Field tests with bulbs treated in silver nitrate-potassium cyanide solutions resulted in the reduction of infection from 26.8 to 1%, a 96% control, and no evidence of injury in the foliage or bloom was detected.

Little attention has been paid to the sterilization of narcissus bulbs with solutions of chemicals owing to the internal location of the principal parasites, the bulb nematode *Tylenchus dipsaci* Kuhn, and the larvae of the bulb fly *Merodon equestris* Fab. The ordinary immersion treatments had little effect, owing to the failure of the disinfectant to reach the bulb parts invaded by the parasites. Longford (1) has recently advocated a method of forcing the sterilizing liquid into the bulbs, by immersing the bulbs in a sealed container and by creating a vacuum over the immersed bulbs. He claimed that the subsequent release of the vacuum forced the sterilizing liquid, a formalin solution, into the bulbs. According to Mackie and Milbrath (2), this method of forcing sterilizing liquids into bulbs is not new. Apparently it was investigated in California in 1922, but was abandoned as having little promise. However, our investigations indicate that the vacuum treatment induces an effective penetration of the sterilizing fluids in the case of narcissus bulbs.

The hot water treatment as developed by Ramsbottom (3), Van Slogteren (4) and others, has been the only satisfactory commercial method of destroying the bulb nematode and the fly larvae in narcissus bulbs. With the object of developing an alternative method, sterilization by immersion in solutions of chemicals *in vacuo* has been investigated.

Experimental

In the initial small-scale experiments a Fruehling and Schultz vacuum desiccator and a Nelson laboratory vacuum pump were used. The de-

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siccator held approximately 50 bulbs and 5 litres of solution and could be evacuated to 3 in. of mercury, in ten minutes. In the semicommercial-scale experiments a pressure cooker of 150-gallon capacity was used. It was equipped with a vacuum gauge and approximately 500 lb. of bulbs could be submerged in 100 gal. of solution therein. With the Nelson laboratory pump this container filled with submerged bulbs could be evacuated to 5 in. of mercury in 30 min. The release of a vacuum of 5 in. of mercury appeared to be sufficient to force the liquid into the tissue invaded by the nematode and bulb fly larvae. The penetration was studied by immersing the bulbs in a solution of methyl violet in the vacuum desiccator. Bulbs left in the solution for 20 min. after the release of the vacuum (total time of immersion 30 min.) were compared with a 3½-hour ordinary immersion and the comparison is illustrated in Fig. 1, A and B. The difference in penetration was striking. The evacuation process forced the dye in between practically every scale from top to bottom and although the ordinary immersion was over a period seven times as long, sections of the immersed bulbs proved that there was little dye penetration.

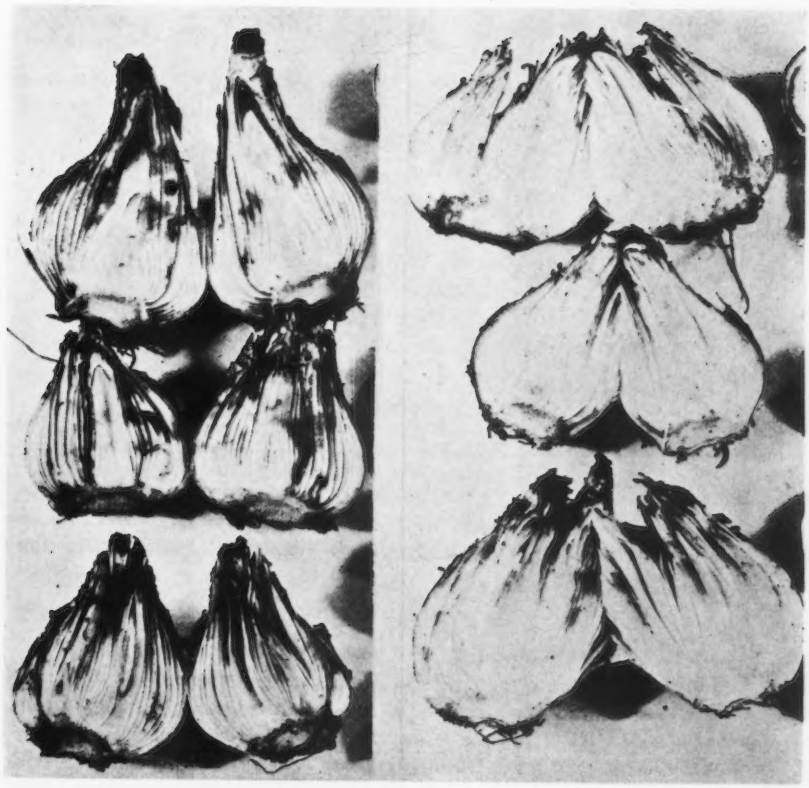
Toxicity of Chemicals Towards Nematodes

The tolerance of living nematodes to various chemicals was approximately determined by increasing the concentration of the chemical in a water suspension of living nematodes to the point where motility ceased within two hours. By this means and by observing the influence of the chemicals upon the subsequent growth of treated bulbs, the following chemicals were discarded as having little promise, because they were either non-toxic to the nematodes or were decidedly toxic to the bulb tissue at the lethal concentrations: ammonium oxalate, ammonium sulphate, boric acid, calcium chloride, copper acetate, copper sulphate, ferric sulphate, hexyl resorcinol, lead acetate, lime (chlorinated), malachite green, mercuric chloride, mercuric nitrate, phenol, potassium bisulphite, potassium chlorate, potassium cyanide, potassium dichromate, potassium polysulphide, sodium benzoate, sodium borate, sodium ferrocyanide, sodium fluoride, sodium thiosulphate, sulphite waste liquor, tannic acid, urea, and zinc chloride.

The following chemicals appeared to be lethal to nematodes at the recorded concentrations: acetic acid, 3.0; beechwood creosote, 0.5; chlorox (5% sodium hypochlorite), 10.0; formalin, 3.0; mercuric cyanide, 1.0; potassium cyanide, 0.3; potassium permanganate, 3.0; potassium bisulphite, 5.0; silver nitrate, 0.05; sodium nitrate, 5.0; sodium sulphide, 0.6%.

Pot Experiments in 1931

Six of the most promising solutions were further tested. Lots of 25 bulbs were passed through three dilutions of each chemical and subsequently planted in six-inch pots in a greenhouse. The influence of each dilution was observed in the foliage, root and bloom development, as well as upon the parasitic nematodes and fly larvae. As a check, a representative lot was run



A. B.

FIG. 1. *Methyl violet penetration as influenced by the evacuation process and ordinary immersion. A. Evacuation process; B. Ordinary immersion.*

through the standard hot water treatment for nematode control, namely, a three-hour immersion in a water bath held at 112°F. The results are given in Table I.

TABLE I

EFFECT OF CHEMICAL TREATMENT OF DISEASED NARCISSUS BULBS BY THE EVACUATION PROCESS ON THE DEVELOPMENT OF THE BULB AND THE NEMATODE PARASITES

Treatment	Plants with apparently normal			Plants in which nematodes survived
	Foliage %	Root %	Bloom %	%
Untreated	88	56	52	48
Hot water, 3 hr. 112°F.	84	84	40	36
Sodium sulphide 1.5%	40	44	32	0
1.0%	55	65	45	20
0.5%	84	76	60	40
Chlorox (5% NaOC1) 16%	75	52	48	32
Silver nitrate 0.15%	68	64	52	0
0.10%	75	75	65	0
0.05%	80	88	64	4
Potassium cyanide 1.5%	8	0	0	Bulbs decayed
1.0%	40	36	16	4
0.5%	56	56	44	32
Formalin 2.0%	24	16	20	8
1.0%	15	15	15	20
0.5%	32	36	20	24
Acetic acid 1.5%	20	5	10	Bulbs decayed

The results suggested that silver nitrate at 0.05% to 0.15% was a satisfactory lethal agent for nematodes in narcissus bulbs. The other five chemicals either failed to destroy the nematodes or were extremely toxic to the host bulbs.

The Instability of Silver Nitrate

The instability of silver nitrate in a solution proved to be the limiting factor in the commercial adoption of this chemical as a disinfectant. The original results could be duplicated only when clean bulbs were used and when the silver nitrate was dissolved in water low in chlorides. When a lot of dirty bulbs were run through the semicommercial set-up, the silver salt rapidly and completely disappeared from the solution and the immersion *in vacuo* was not effective at the intermediate concentration, namely 0.1%.

In order to prevent the silver from being thrown out of solution as insoluble chlorides and other insoluble compounds through the interaction of the silver nitrate with the salts normally present in the dirt on the bulbs and in tap water, a combination of silver nitrate and potassium cyanide was used in the ratio of one to three by weight. In this solution the silver is present largely

as part of the complex Ag $(CN)_2^-$ ion and silver in this form is not thrown out of solution by chlorides. The large excess of potassium cyanide ensures stability even when a considerable part of the cyanide is lost through reactions with the dirt on the bulbs.

The solution adopted as a standard contained silver nitrate 0.05 and potassium cyanide 0.15% ($\frac{1}{2}$ and $1\frac{1}{2}$ lb. respectively, in 100 gal. of water). By adding soil to this solution in quantities much in excess of what would normally come in contact with the solution through the treatment of dirty bulbs, the loss of silver was insignificant. In practice about one-tenth of the original volume is lost when each lot of wet bulbs is taken from the silver nitrate-potassium cyanide solution. The replacement of this volume with fresh solution of standard strength is all that appears necessary to maintain an effective concentration.

Four lots of abnormally dirty bulbs were put through the same solution in succession adding only sufficient fresh solution to maintain the original volume. The kill of both nematodes and bulb fly larvae appeared to be as effective in the last lot as in the first. A fraction of the bulbs so treated were examined 14 days after the treatment, and no living nematodes or bulb fly larvae were found.

A special lot of bulbs containing fly larvae (*Merodon equestris*) were put through the standard silver nitrate-potassium cyanide evacuation process, and the larvae were examined by Mr. W. Downes*. He found that the treatment effected a 100% mortality.

Bulbs treated by immersion in solutions of chemicals *in vacuo*, like those treated with hot water, should be thoroughly drained and partly dried before planting in heavy or wet soils, or injury may follow through oxygen deficiency.

Field Experiments

Nematode-infested King Alfred bulbs supplied by a commercial grower were used in the field plot experiments. After treatment they were planted in $\frac{1}{200}$-acre plots and trenches were dug between the plots to prevent the migration of the nematodes from one plot to another under distinctive treatment. The data are presented in Tables II and III.

The silver nitrate-potassium cyanide solutions at standard, two and three times standard strength markedly reduced the amount of nematode infection compared with the untreated as shown by the reduction in the number of plants bearing "spikkels". However, these solutions do not appear to be quite as effective as hot water. The apparent perfect control by the hot water may be due to the presence of 0.5% formalin or to the fact that the temperature was maintained at 114°F., two degrees higher than standard. Previous experiments showed that the King Alfred variety is more tolerant to high temperatures than most varieties of narcissus. As expected, silver

*The authors are pleased to acknowledge the assistance of Mr. W. Downes, Dominion Entomological Branch, Victoria, B.C.

TABLE II

NEMATODE CONTROL AS EFFECTED BY FORCING SOLUTIONS INTO THE BULBS BY IMMERSION *in vacuo* AND BY A MODIFIED HOT WATER TREATMENT

Treatment	Time of treatment; weeks after lifting	Bulbs planted (King Alfred)	No. and percent with "spikkels"		Control relative to untreated
		Number	No.	%	%
Untreated		250	67	26.8	
Silver nitrate 0.1%	2	236	17	7.1	
	3	250	19	7.6	
	4	236	21	8.9	
	5	250	9	3.6	
	7	250	9	3 6	
		1222	75	Av. 6.1	77
Potassium cyanide 0.5%	2	125	15	12.0	
	3	118	14	11.8	
	4	125	26	20.4	
	5	125	10	8.0	
		493	65	Av. 13.2	50
Silver nitrate 0.5% Potassium cyanide .15%	7	100	1	1.0	
Silver nitrate 0.1% Potassium cyanide 0.3%	7	900	6	0.6	
Silver nitrate 0.15% Potassium cyanide 0.45%	7	200	5	2.5	
		1200	12	Av. 1.0	96
Sodium sulphide 1.0%	2	125	20	16.0	
	3	119	32	26.8	
	4	125	35	28.0	
	5	125	31	24.0	
		494	118	Av. 23.7	11
Hot water and 0.5% formalin, 3 hr. at 114°F.	3½	250	0	0	100

nitrate alone gave some measure of control, for clean bulbs were used, but the silver nitrate solution was not as effective as when combined with potassium cyanide, and no other solution under test gave promise.

To determine whether any deterioration of the silver nitrate-potassium cyanide solution took place, a series of 14 lots of uncleaned bulbs, a total of 700, were run in succession through five litres of solution adding only enough fresh solution to compensate for the loss of liquid absorbed by the bulbs. A total of three litres of solution was absorbed. The results are given in

Table III, and indicate that the efficiency of the solution remains constant when only sufficient fresh solution is added to maintain the necessary volume to cover the bulbs.

TABLE III

THE INFLUENCE OF SUCCESSIVE IMMERSION OF BULB LOTS IN THE SAME SOLUTION OF SILVER NITRATE-POTASSIUM CYANIDE

Chemical solution	Sequence of lots	Number of bulbs treated	Number of plants with spikkels	Per cent diseased
Check—untreated		250	67	26.8
Silver nitrate 0.1% and potassium cyanide 0.3%	1st	50	0	0
	2nd	50	1	2
	3rd	50	0	0
	4th	50	0	0
	5th	50	0	0
	6th	50	0	0
	7th	50	0	0
	8th	50	0	0
	9th	50	0	0
	10th	50	0	0
	11th	50	1	2
	12th	50	0	0
	13th	50	1	2
	14th	50	0	0
		700	3	Av. 0.4

NOTE:—*A vacuum desiccator was used for this experiment and it was necessary only to supplement the initial five litres of solution with one litre after the 7th, 9th and 12th lots, to keep the bulbs covered.*

No evidence of injury to the bulbs could be detected as induced by the silver nitrate-potassium cyanide solution at standard, two and three times standard concentrations. The measurements of the foliage and bloom showed that in these experiments there was no significant difference between the plants from treated bulbs and the healthy plants in the untreated plots. The only difference noticed was in the case of bulbs in an advanced state of decay through nematode infestation. Untreated, such bulbs would produce a feeble growth but after treatment they would not grow at all.

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NEMATODE INFESTATION SYMPTOMS ON BARLEY AS A MEANS OF DETERMINING THE EFFICIENCY OF CHEMICALS AS LETHAL AGENTS AGAINST *TYLENCHUS DIPSACI* KUHN[1]

By W. Newton,[2] R. J. Hastings[3] and J. E. Bosher[3]

Abstract

Barley is suggested as a detector crop for the presence of living nematodes, *Tylenchus dipsaci*, in soil, owing to rapid development of nematode disease symptoms on barley.

A satisfactory source of inoculum consists of the white masses of coiled nematodes that can be seen when the basal plate is removed from badly diseased narcissus bulbs. These masses remain viable for six months or longer when removed from the bulbs.

Low temperatures and high light conditions favor the development of the nematode disease symptoms in barley seedlings, after nematodes are transferred from narcissus bulb to autoclaved soil planted with barley. Such barley seedlings were broad-leaved and stocky. Under low light and high temperatures, conditions that favor the development of spindly seedlings, the nematode disease symptoms are inconspicuous or absent.

Few chemicals appear to be lethal to the bulb nematode. Of 100 tested only phenol, silver nitrate, and potassium or sodium bisulphite were lethal at dilute concentrations.

In a recent paper (1) the writers reported that when barley, wheat and oats are planted in pots in soil inoculated with the narcissus bulb nematode *Tylenchus dipsaci* Kuhn, conspicuous symptoms of the nematode disease rapidly developed in the seedlings. The writers have since found that nematode infestation symptoms develop in fall rye seedlings when likewise inoculated. Owing to the fact that the symptoms were most conspicuous in the barley seedlings it was thought that after treatment with various chemicals the presence of living nematodes might be conveniently determined by transferring the nematode suspensions to pots of soil planted with barley. Barley has many advantages over narcissus bulbs in the detection of living nematodes. In contrast with narcissus bulbs the chances are small that barley seed is ever naturally infected with the narcissus bulb nematode. Furthermore, the cost is much lower and data can be obtained in a much shorter time. One month from the time the barley is planted the symptoms of nematode infection are usually evident. Moreover, unlike the use of narcissus bulbs the use of barley enables the experimenter to conduct a series of experiments regardless of season, for crops of barley seedlings can be grown in rapid succession throughout the year.

[1] *Manuscript received May 8, 1933.*

Contribution from the Dominion Laboratory of Plant Pathology, Saanichton, British Columbia, Canada.

[2] *Plant Pathologist-in-charge, Dominion Laboratory of Plant Pathology, Saanichton, British Columbia.*

[3] *Plant Disease Investigators, Dominion Laboratory of Plant Pathology, Saanichton, British Columbia.*

Inoculum

Macerated tissue of soft bulbs severely infested with *Tylenchus dipsaci* were used in the preliminary experiments as the source of inoculum. This inoculum proved to be unsatisfactory owing to the frequent transfers of *Fusarium* species and other parasites that cause damping off of the barley seedlings. In these experiments practically pure cultures of nematodes were used. The inoculum was obtained by selecting severely infected bulbs that had been kept in storage for at least two months. These bulbs were soft and partly decayed and when the basal plates were removed, white cottony masses (Fig. 1) of what proved to be thousands of coiled dormant nematodes (Fig. 2) could be seen with the naked eye. These masses were pricked off and maintained as inoculum, and when stored in cork-stoppered bottles for six months the nematodes therefrom would revive in about three hours when suspended in tap water. A needle transfer from the stock inoculum usually yielded over 1,000 nematodes. These, revived in water, were used throughout these experiments and were found capable of infecting barley, wheat, oat and rye seedlings as well as disease-free narcissus bulbs.

Symptoms of Nematode Attack on Barley Seedlings

The symptoms of nematode infestation on barley seedlings are easy to recognize. The leaves show definite white spots, mainly along the midrib (Fig. 3).* Sometimes these are slightly raised, and occasionally a leaf is markedly deformed. Infestation shows up early, the symptoms usually being conspicuous four weeks after seeding.

Experimental

Conditions Favoring Infestation and Development of Symptoms

Barley seeds were sown thickly in five-inch pots containing steam-sterilized soil (1 hr. at 25 lb. pressure), and were inoculated with 100 cc. of water containing over 1,000 nematodes. The pot cultures were then allowed to develop in an ordinary greenhouse, each lot under distinct but variable conditions of light and temperature. Observations were made when the seedlings had reached 8 to 16 cm. in height. The conditions of growth and the results are given in Table I.

Nematode-infestation symptoms are conspicuous in barley seedlings grown under an environment that favors the development of stocky plants with broad fully expanded leaves, *e.g.*, full light and low temperatures. Under such an environment the symptoms can be readily detected four weeks after seeding when the plants are approximately 10 cm. high. When the seedlings are grown in the semi-shade or diffused light at low temperatures or under rapid growing conditions induced by high temperatures, spindly plants develop whereon the symptoms of nematode infestation are seldom found.

**See also Reference 1, Fig. 1.*

PLATE I

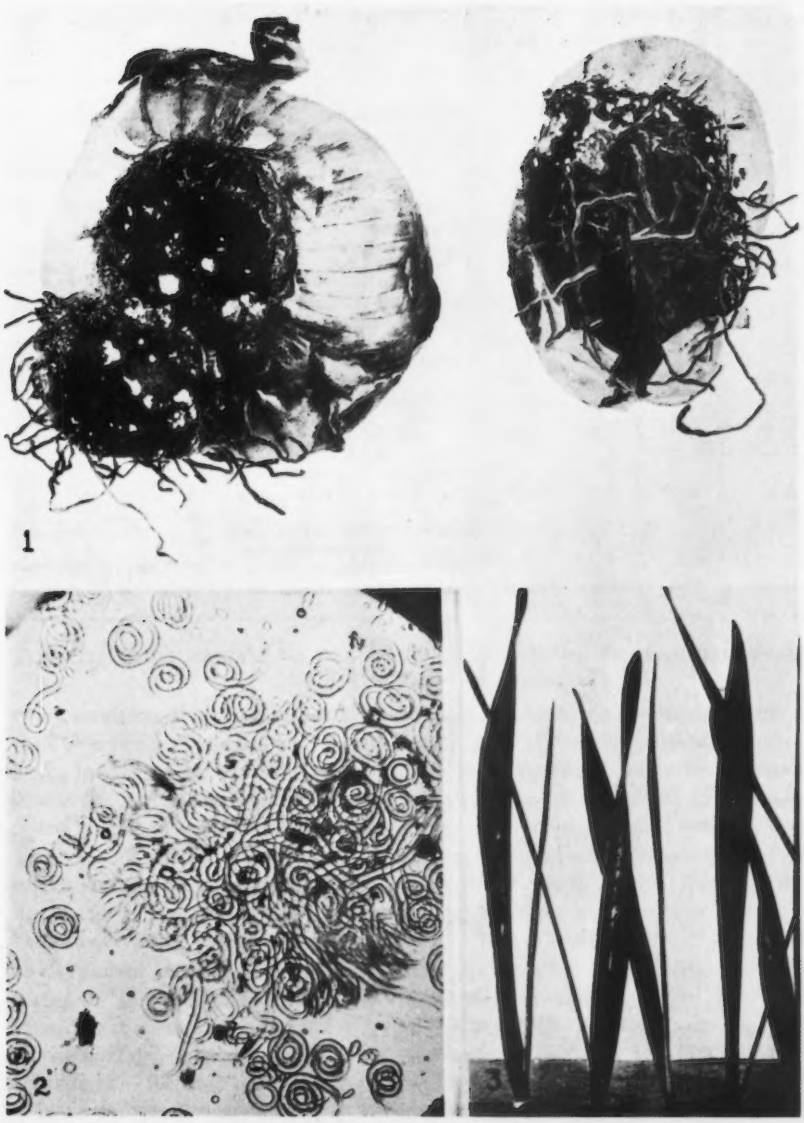

FIG. 1. *Bulbs with nematode clusters in the basal plate and on the old roots.* FIG. 2. *Part of a cluster of nematodes in a drop of water* × *50.* FIG. 3. *Barley seedlings showing symptoms of infection by the bulb nematode.*

TABLE I

THE PERCENTAGE OF BARLEY PLANTS INFESTED AS INFLUENCED BY THE ENVIRONMENT OR SEEDLING TYPE

Date of experiment and source of inoculum	Environment and seedling type	Type of plant			Percentage infected
		Av. height of plants, cm.	Av. length of stem, cm.	Av. width of leaves, cm.	
Oct. 13-Nov. 2 KING ALFRED BULBS Saanichton, B.C.	Pots in unheated greenhouse, temperature range 43-89° F. full light: stocky plants	12	2.0	0.7	17.7
Nov. 17-Dec. 2 SIR WATKINS BULBS Gordon Head, B.C.	Pots in closed case in greenhouse, temperature range 60-85° F. diffused light: spindly plants	15	4.0	0.4	1.3
Jan. 3-Jan. 29 SIR WATKINS BULBS Gordon Head, B.C.	Pots in unheated chamber off greenhouse, temperature range 35-85° F.— A. Full light: stocky plants	10	1.5	0.7	20.5
	B. Semi-shade, pots resting on boards: spindly plants	15	4.5	0.5	13.3
	C. Semi-shade, pots plunged in peat: spindly plants	15.5	4.5	0.4	6.6
Feb. 3-Feb. 28 PRINCEPS BULBS Colwood, B.C.	Pots in unheated chamber off greenhouse, temperature range 32-80° F. full light: stocky plants	9.0	1.0	0.6	56.0

TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS DETERMINED BY THE BARLEY INDEX METHOD

By studying the distribution of a dye solution the writers recently concluded that solutions of chemicals can be forced into the regions of the narcissus bulbs inhabited by the bulb nematode by immersion *in vacuo*. Furthermore, it was found that immersion *in vacuo* in a combination solution of potassium cyanide and silver nitrate was a promising control for the bulb nematode (4). The present study is part of a search for agents lethal to nematodes and relatively non-toxic to plant tissue at the lethal concentrations. The standard hot water treatment for bulb sterilization, in vogue for 15 years, has lately been observed to yield inconsistent results. The treatment, it will be recalled, as developed by Ramsbottom (5) required a three hours' submergence in hot water at 110° F., and a retest of this treatment under precise control has shown that nematodes were alive in old lesions when the bulbs were examined in the spring. There appears to be an urgent need for a modification of the treatment. Raising the temperature of the hot water is effective in ensuring a more complete destruction of the nematodes, but the risk to the health of

the bulb is often great. An alternative is to add a supplemental killing agent to the hot water, but the present knowledge of lethal agents for the bulb nematode is very limited. Apart from the writers' report that immersion in silver nitrate-potassium cyanide solution *in vacuo* is effective, they can find reference to only one other chemical agent: *viz.*, formalin. This was recommended by Longford (3) as a cold dip applied *in vacuo*, and by the United States Department of Agriculture (6, 7) as a supplement to the standard hot water treatment, to prevent the survival of nematodes that escape into the hot water. However, Johnson and Godfrey (2) report chloropicrin as an effective soil fumigant against Root Knot, hence this chemical might find application as an agent for the destruction of the bulb nematode.

Experimental

The toxicity of the following chemicals was determined in the manner outlined above, except that the nematodes were suspended in a chemical solution instead of water. If they were destroyed by the chemical there could, of coûrse, be no infestation. On the other hand, if the chemical were not lethal, the nematodes would probably be as capable of infecting barley as when suspended in water. The tests were made in triplicate. One cubic centimetre of each prepared solution was placed in a test tube. A needle transfer of the nematodes was made from the stock inoculum into each of these test tubes, and after contact with the solution for two hours each test tube was filled with water and emptied into a three-inch pot of sterilized soil previously seeded to barley. The pots were then thoroughly watered and kept under the optimum conditions for infection as described above. The observations were recorded from three to four weeks after seeding and are presented as Table II.

TABLE II

TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS REVEALED BY THE ABILITY OF THE IMMERSED NEMATODES TO INFECT BARLEY

Chemical	Concentration				Chemical	Concentration			
	Saturated solution	2%	1%	0.5%		Saturated solution	2%	1%	0.5%
1. Acetamide		N			11. Ammonium carbonate		N		
2. Acid benzoic	N				12. Ammonium chloride		N		
3. Acid boric		X			13. Ammonium citrate		N	N	N
4. Acid carbolic (phenol)		O	O	O	14. Ammonium oxalate		N		
5. Acid molybdic		N			15. Ammonium phosphate				
6. Acid oxalic		N			(secondary)		N		
7. Acid picric	N				16. Ammonium sulphate		N		
8. Acid pyrogallic		N			17. Ammonium tartrate		N	N	N
9. Acid salicylic	N				18. Amyl acetate	N			
10. Acid sulphanilic	N				19. Barium chloride		N	N	N

Symbols—N, *plants infected—solution non-toxic to nematodes.*
X, *chemicals toxic to barley, indefinite results.*
O, *healthy plants, solution lethal to nematodes.*

TABLE II—*Concluded*

TOXICITY OF CHEMICALS TO THE BULB NEMATODE AS REVEALED BY THE ABILITY OF THE IMMERSED NEMATODES TO INFECT BARLEY

Chemical	Concentration				Chemical	Concentration			
	Saturated solution	2%	1%	0.5%		Saturated solution	2%	1%	0.5%
20. Brucine	N				62. Potassium phosphate (secondary)		N	N	N
21. Cadmium sulphate		N			63. Potassium phosphate (tertiary)		N	N	N
22. Cresol		N			64. Potassium persulphate		N		
23. Creosote	N				65. Potassium silver cyanide		O		
24. Cupric acetate		N	N	N	66. Potassium sulphate		N		
25. Cupric nitrate		O	N	N	67. Potassium thiocyanate		N		
26. Cupric sulphate		O	N	N	68. Quinine	N			
27. Diphenylamine	N				69. Resorcinol		N		
28. Dyes—Auramine O.S.		N			70. Saponin		N		
29. Fluoresceine L.T.S.		N			71. Silver nitrate		O	O	O
30. Indian blue 2 R.S.		N			72. Sodium acetate		N		
31. Magenta P.S.		N			73. Sodium benzoate		N		
32. Malachite green		N			74. Sodium bicarbonate		N		
33. Rhodamine		N			75. Sodium bisulphite		O	O	O
34. Ethyl acetate		N			76. Sodium borate		N		
35. Ferric ammonium sulphate		O	O	N	77. Sodium carbonate		N		
36. Ferric chloride		N			78. Sodium chloride		N		
37. Ferrous ammonium sulphate		N			79. Sodium ferrocyanide		N		
38. Ferrous sulphate		N			80. Sodium fluoride		N		
39. Formalin		O	N	N	81. Sodium fluosilicate (Consolidated Mining and Smelting Co.)	N			
40. Glycerine		N			82. Sodium fluosilicate (Virginia-Carolina Chemical Co.)	N			
41. Hydrochinone		N			83. Sodium hydroxide		O		
42. Lead acetate		N			84. Sodium nitrite		N		
43. Lead carbonate	N				85. Sodium nitroprusside		N		
44. Magnesium phosphate	N				86. Sodium oxalate		N		
45. Magnesium sulphate		N			87. Sodium peroxide		O		
46. Mercuric chloride		O	O	N	88. Sodium potassium tartrate		N		
47. Mercuric cyanide		X			89. Sodium phosphate		N		
48. Mercuric nitrate		O	N	N	90. Sodium salicylate		X		
49. Naphthylamine, alpha	N				91. Sodium sulphate		N		
50. Naphthol, beta	N				92. Sodium sulphite		N		
51. Piperine	N				93. Sodium thiocyanate		N		
52. Potassium acetate		N	N	N	94. Sodium thiosulphate		N		
53. Potassium bichromate		N			95. Stannous chloride		N		
54. Potassium bisulphite		O	O	O	96. Thymol	N			
55. Potassium chlorate		X			97. Urea		N		
56. Potassium nitrate		N			98. Vanillin		N		
57. Potassium iodate		X			99. Zinc chloride		N		
58. Potassium iodide		X			100. Cheshunt Compound		O	N	N
59. Chinosol		N							
60. Potassium permanganate		N	N	N					
61. Potassium phosphate (primary)		N	O	N					

The most promising chemicals appeared to be phenol, potassium and sodium bisulphite and silver nitrate. In general, the work revealed that the bulb nematodes are difficult to destroy by chemicals, even by substances commonly used as fungicides and insecticides. An experimental search for lethal agents is under way among the less common chemicals.

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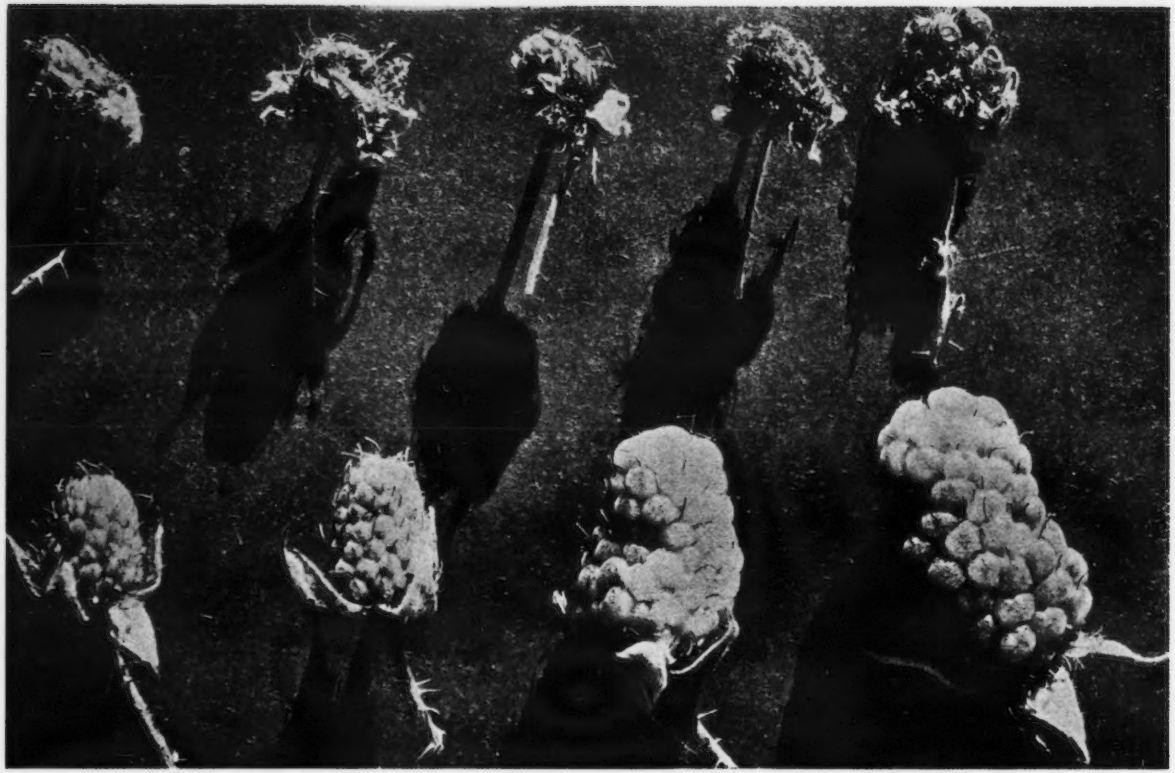

FIG. 1 *Disease of loganberry caused by Hapalosphaeria deformans Syd. Upper—Diseased. Lower—healthy.*

PLATE I

ANTHER AND STIGMA BLIGHT OF LOGANBERRY[1]

By J. Dearness[2] and W. R. Foster[3]

Abstract

A new disease of loganberry, an anther and stigma blight, is reported from British Columbia. This disease is caused by *Hapalosphaeria deformans* Syd., a new fungus for North America. The fungus prevents pollination of a number of the drupelets and a deformation of the fruit results.

Introduction

In the spring of 1932, the junior author's attention was directed to deformed loganberries with a white fungus on the anthers and stigmas (Fig. 1). The fungus was also found on the petals which were touching the anthers. The estimated loss from the fungus in a number of fields in Royal Oak and Gordon Head districts, Vancouver Island, was 3 to 5%. Growers were alarmed enough to send reports and samples of the injury to the laboratory. The authors collaborated in a study of the fungus.

Etiology

It was found that Sydow (2) described the hyphomycetous form of this fungus with superficial mycelium and isthmus-connected globoid conidia on *Rubus caesius* L. in 1907 and named it *Paepalopsis deformans* Syd. In the following year Diedicke studied the disease further and inferred that the *Paepalopsis* in this case is a stage or form of a sphaeropsid fungus found chiefly in the anthers. Sydow concurred in this inference. Diedicke (1) expressed the surprise both of himself and Sydow that the presumptive hyphomycete is also a good sphaeropsid. Upon the discovery, Sydow founded a new genus which he called *Hapalosphaeria* on the type species *H. deformans* Syd.

The fungus in the distorted flowers of the loganberry, *Rubus loganobaccus* Bailey agrees with the description of the above named species. Both the curious hyphal form and the sphaeropsid were present in at least one opening flower bud examined by the senior author. In one anther there were no fewer than 56 pycnidia each with its multitude of smooth, colorless globoid spores 3–4 μ in diameter.

The fungus prevents the pollination of a number of the drupelets and a deformation of the fruit results, as shown in Fig. 1. The presence of the disease in the old world and its first reported appearance on the Pacific Coast of America give ground for the supposition that it is an Asiatic parasite of the raspberry which has invaded this continent by the Northwest.

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[1] *Manuscript received April 10, 1933.*
Contribution from the Provincial Laboratory of Plant Pathology, Saanichton, British Columbia, Canada, with financial assistance from the National Research Council of Canada.

[2] *Mycologist, London, Ontario.*
[3] *Assistant Plant Pathologist, British Columbia Department of Agriculture.*

CHEESE-RIPENING STUDIES[1]

Casein-splitting Abilities of Lactic Acid Bacteria

By Blythe Alfred Eagles[2] and Wilfrid Sadler[3]

Abstract

The casein-splitting ability of each of seven cultures isolated from Kingston cheese has been studied. The cultures are Gram positive non-gelatin-liquefying coccus forms; some appear as chains in young milk culture, others as pairs. The methods of Orla-Jensen and Wasteneys and Borsook were used.

It is shown that: (a) Cultures EMB_2 166 and 168 fail to attack casein; (b) the casein-splitting abilities of cultures EMB_1 131, 133, 173, 195 and EMB_2 173 are established and well-defined; (c) the type of proteolysis characteristic of cultures EMB_1 173 and 195 leads to the formation of large amounts of the simpler degradation products, whereas the proteolytic breakdown characteristic of cultures EMB_1 131, 133 and EMB_2 173 is not as complete; (d) culture EMB_2 173 is distinct in its casein-splitting abilities, as is evident when the results obtained by the two methods are compared; (e) unable to attack casein, cultures EMB_2 166 and 168 attack the non-protein-nitrogenous components of milk; (d) culture EMB_2 166 may be capable of protein synthesizing action.

It has been shown by Orla-Jensen (4) in his studies on the lactic acid bacteria that the casein-splitting ability of an organism may be defined by determining the soluble nitrogen and amino nitrogen after incubation of the culture in chalk milk at the appropriate temperature for a period of six weeks. From his work it is to be seen that some organisms fail to hydrolyze the milk protein; that certain organisms produce an appreciable amount of soluble nitrogen, but amino nitrogen in a limited measure only; and that certain organisms attack the milk protein in such manner that not only is a considerable amount of soluble nitrogen formed, but also a relatively large amount of amino nitrogen. Not only have the writers employed the methods of Orla-Jensen in determining the casein-splitting abilities of lactic acid bacteria but also have used the method of Wasteneys and Borsook (9)—the method developed by them for the fractional analysis of incomplete protein hydrolysates.

Methods

For the determination of total nitrogen, soluble nitrogen and amino nitrogen after the manner of Orla-Jensen the writers have followed the methods used by him in his studies on the lactic acid bacteria (1, 4, 5, 6). In determining the proteose nitrogen, peptone nitrogen and subpeptone nitrogen after the manner of Wasteneys and Borsook the procedure has been almost identical with that described by the writers (1).

[1] *Manuscript received May 1, 1933.*

These studies were provided for by a Research Fund established jointly by the Empire Marketing Board and the University of British Columbia. As from Feb. 1 to Sept. 30, 1933, work proceeds under a joint grant of the Empire Marketing Board and the National Research Council of Canada, laboratory facilities being found by the University of British Columbia.

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[3] *Professor of Dairying, University of British Columbia, Vancouver, British Columbia, Canada.*

In the work on certain cultures it was found impossible to filter the solution when following the procedure for determining proteose, because large quantities of what is presumably a soap salted out. Consequently in working on these cultures one departure from the technique has had to be made. The solution was filtered after it had stood for 15 min.—instead of for one hour, the length of time defined in the original method (9)—two grams of anhydrous sodium sulphate was added to the filtrate and the mixture was then kept at 35° C. for 45 min. before filtering in a water-jacketed filter maintained at 33° C.

Experimental

Employing the methods cited (1, 4, 5, 6, 9), a study of the casein-splitting abilities of seven lactic acid producing coccus forms has been undertaken. All the strains were recovered from Kingston cheese (7). Cultures EMB_1 131, 133, 173 and 195 were isolated from a Kingston cheese of the "make" of Dec. 4, 1929—at the time the cheese was two weeks old and characteristic of the type (7). Cultures EMB_2 166, 168 and 173 were isolated from another Kingston cheese of the make of Dec. 4, 1929—at the time the cheese was two months old (7). The seven cultures are Gram positive non-gelatin-liquefying coccus forms: some appear as chains in young milk culture and some are to be seen as pairs: the total titratable acidity produced by each strain in milk and in milk enriched with yeast extract has been determined repeatedly (7). The influence of defined nitrogen sources on the sugar-fermenting abilities of certain of these cultures has been studied (7, 8) and the effect on their sugar-fermenting abilities of enriching peptic casein digest broth with different concentrations of yeast extract has been determined (7).

The results of the determinations of the casein-splitting abilities of the seven cultures are given below in Table I.

Discussion

It is to be seen that there is considerable variation in the total nitrogen content of the milk employed as controls for the determinations.

In the case of the controls the soluble nitrogen figure is always considerably higher than is the non-protein-nitrogen figure (Table I). Consequently, inasmuch as the trichloracetic acid filtrate is made up of non-protein-nitrogenous compounds only, that amount of nitrogen obtained on subtraction of the non-protein-nitrogen figure from the corresponding soluble nitrogen figure, represents "something" of a greater complexity than is proteose, "something" of a complexity approaching that of a complete protein.

It may be suggested that this "something" present in the control milk—milk autoclaved with calcium carbonate—is formed by the autoclaving process from a "something" physically more simple to be found in fresh skim milk.

Enquiring into the validity of this hypothesis, a quantity of fresh skim milk was divided into three equal parts: one aliquot remained untreated; the other two portions were autoclaved at 14 lb. pressure for 25 min. after the

TABLE I

CASEIN-SPLITTING ABILITIES OF LACTIC ACID BACTERIA. NITROGEN DISTRIBUTION IN CHALK MILK CULTURE

Culture number	Isolated from	Morphology	Total nitrogen	Nitrogen—Mgm. per 100 cc. milk						Nitrogen formed—Mgm. per 100 cc. milk						Nitrogen formed—% of total nitrogen					
				Soluble nitrogen	Amino nitrogen	Non-protein nitrogen	Proteose nitrogen	Peptone nitrogen	Subpeptone nitrogen	Soluble nitrogen	Amino nitrogen	Non-protein nitrogen	Proteose nitrogen	Peptone nitrogen	Subpeptone nitrogen	Soluble nitrogen	Amino nitrogen	Non-protein nitrogen	Proteose nitrogen	Peptone nitrogen	Subpeptone nitrogen
Control			555.2	91.10	31.80	48.72	3.73	23.83	21.16												
EMB$_1$ 131	Kingston cheese, 13 days old	Coccus		155.60	37.35	149.28	14.16	64.06	71.06	64.50	5.55	100.56	10.43	40.23	49.90	11.62	1.00	18.11	1.88	7.24	8.99
EMB$_1$ 133	Kingston cheese, 13 days old	Coccus		138.80	34.80	129.40	18.22	53.52	57.66	47.70	3.00	80.68	14.49	29.69	36.50	8.41	0.54	14.53	2.61	5.35	6.57
Control			505.8	75.80	19.20	30.92	1.13	25.45	4.34												
EMB$_1$ 173	Kingston cheese, 13 days old	Coccus		137.00	73.80	132.16	23.42	42.20	66.54	61.20	54.60	101.24	22.29	16.75	62.20	12.10	10.80	20.02	4.41	3.31	12.30
EMB$_1$ 195	Kingston cheese, 13 days old	Coccus		113.80	60.60	114.24	18.04	46.07	50.13	38.00	41.40	83.32	16.91	20.62	45.79	7.52	8.19	16.47	3.34	4.07	9.06
EMB$_2$ 168	Kingston cheese, 2 months old	Coccus		76.00	20.70	43.96	4.24	23.81	15.91	0.20	1.50	13.04	3.11	−1.64	11.57	0.04	1.13	2.58	0.62	−0.33	2.29
Control			523.4	85.40	28.20	42.72	2.82	16.32	23.56												
EMB$_1$ 173	Kingston cheese, 13 days old	Coccus		154.50	76.20	144.24	13.37	57.09	73.78	69.10	48.00	101.52	10.55	40.77	50.22	13.21	9.18	19.40	2.01	7.78	9.61
EMB$_1$ 195	Kingston cheese, 13 days old	Coccus		157.50	83.10	149.20	13.54	55.06	80.60	72.10	54.90	106.48	10.72	38.74	57.04	13.78	10.50	20.34	2.05	7.40	10.89
EMB$_2$ 166	Kingston cheese, 2 months old	Coccus		75.80	16.50	37.68	5.76	4.64	27.28	−9.60	−11.70	−5.04	2.94	−11.68	3.72	−1.83	−2.23	−0.96	0.56	−2.23	0.71
EMB$_2$ 168	Kingston cheese, 2 months old	Coccus		78.50	15.00	43.68	6.18	22.62	14.88	−6.90	−13.20	0.96	3.36	6.30	−8.68	−1.31	−2.48	0.18	0.64	1.20	−1.66
EMB$_2$ 173	Kingston cheese, 2 months old	Coccus		145.00	18.30	93.12	40.72	22.02	30.38	59.60	−9.90	50.40	37.90	5.70	6.82	11.39	−1.89	9.63	7.24	1.08	1.31
Control			526.8	75.00	34.20	43.68	6.18	12.08	25.42												
EMB$_1$ 131	Kingston cheese, 13 days old	Coccus		127.80	66.60	127.68	26.88	52.44	48.36	52.80	32.40	84.00	20.70	40.36	22.94	10.02	6.15	15.95	3.93	7.66	4.36
EMB$_1$ 195	Kingston cheese, 13 days old	Coccus		149.00	90.00	147.60	11.74	64.98	70.68	74.00	55.80	103.92	5.56	52.90	45.26	14.04	10.59	19.73	1.06	10.04	8.59
EMB$_2$ 166	Kingston cheese, 2 months old	Coccus		73.80	33.90	43.92	0.03	12.27	31.62	−1.20	−0.30	0.24	−6.15	0.19	6.20	−0.23	−0.05	0.05	−1.17	0.04	1.18

addition of 3% calcium carbonate to one of them. The soluble nitrogen, the nitrogen of the trichloracetic acid filtrate direct and the non-protein-nitrogen respectively in each aliquot was then determined. The results are given, in mgm. per 100 cc. milk, in Table II.

TABLE II

Milk	Soluble nitrogen	Non-protein nitrogen	Nitrogen of trichloracetic filtrate direct
Milk—fresh	74.00	33.80	68.80
Milk—autoclaved	63.00	36.20	38.80
Milk—autoclaved with $CaCO_3$	68.00	35.04	39.60

The soluble nitrogen figures agree rather closely and the non-protein-nitrogen figures approximate the one the other, Table II. It is equally clear that the latter are considerably lower than the former. The figures for the nitrogen determinations on the trichloracetic acid filtrate direct—that is the filtrate prior to concentration—agree closely with the non-protein-nitrogen figures in the case of milk autoclaved with or without calcium carbonate. On the other hand, in the case of fresh skim milk the nitrogen content of the trichloracetic filtrate direct is quite closely comparable with the soluble nitrogen figure.

It would appear that in being subjected to the autoclaving process the proteins in milk assume a complexity of a nature comparable with the complexity arrived at when subjected to heat as part of the Wasteneys and Borsook procedure for the complete removal of protein by trichloracetic acid.

Whilst it is shown that cultures EMB_2 166 and 168 fail to attack casein, the casein-splitting abilities of cultures EMB_1 131, 133, 173, 195 and EMB_2 173 are established and well defined, Table I.

When the amount of amino nitrogen formed by an organism is taken as an indication of the extent to which the breakdown of casein has been carried, it is shown that the type of proteolysis characteristic of cultures EMB_1 173 and 195 is of a nature leading to the formation of large amounts of the simpler degradation products and that for cultures EMB_1 131, 133 and EMB_2 173 the proteolytic breakdown is not as complete, (Table I).

Applying the method of Wasteneys and Borsook, it is shown that a more detailed defining of the nature and amount of the protein decomposition products—proteose, peptone and subpeptone—in the control milk and in the milk in which the organisms have been grown is obtained. When this method is applied, the striking difference, shown above, in the nature of the proteolytic breakdown brought about by cultures EMB_1 131 and 133 on the one hand and by cultures EMB_1 173 and 195 on the other hand cannot be seen.

Culture EMB_2 173 would appear to be distinct among the casein-splitting strains reported upon. While the amount of soluble nitrogen formed by the

culture is comparable with the amount of soluble nitrogen formed by cultures EMB_1 131, 133, 173 and 195, it is shown that the amount of amino nitrogen formed by culture EMB_2 173 is not comparable with the amount of amino nitrogen formed by any of the other casein-splitting strains—actually the amino nitrogen figure being lower than the amino nitrogen figure of the control, (Table I). The non-protein nitrogen formed approximates the soluble nitrogen formed when the casein-splitting abilities of cultures EMB_1 131, 133, 173 and 195 are considered, whereas in the case of culture EMB_2 173 the non-protein nitrogen formed is considerably less than is the amount of soluble nitrogen formed, the major portion of the non-protein nitrogen consisting of proteose nitrogen—the more complex of the protein-breakdown entities. Thus the distinct nature of the proteolytic ability of culture EMB_2 173 is the more clearly seen when the results obtained by employing the method of Orla-Jensen are compared with the results obtained by using the method of Wasteneys and Borsook, (Table I). If we may interpret the results of the determination of the distribution of protein and protein-breakdown products respectively as an expression of the protein hydrolysis-protein synthesis equilibrium attained by an organism, may it not be that the amount of nitrogen obtained on subtraction of the non-protein-nitrogen figure from the soluble nitrogen figure in the case of culture EMB_2 173 represents "something" of a greater complexity than is proteose, "something" of a complexity approaching that of a complete protein. In so far as its proteolytic ability is concerned culture EMB_2 173 would appear to be quite distinct as compared with the strictly non-casein-splitting forms on the one hand and the active casein-splitting forms on the other hand.

When the results obtained by the method of Wasteneys and Borsook for the non-casein-splitting strains are considered it is shown that cultures EMB_2 166 and 168 are capable of acting upon the non-protein-nitrogenous components of milk and the results obtained for culture EMB_2 168 appear to suggest that the nature of this action is dependent upon the kind of nitrogen moieties to be found in a particular milk (Table I). Further, it is to be seen that for culture EMB_2 166, the non-protein-nitrogen figure is lower than is the non-protein-nitrogen figure of the control, a finding which would seem to indicate protein-synthesizing action—an hypothesis already proposed by Hucker (3) when working with certain lactic-acid-producing coccus forms.

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THE EFFECT OF RADIANT ENERGY ON DIASTASE ACTIVITY[1]

By A. H. Hutchinson[2] and Miriam R. Ashton[3]

Abstract

The effects of radiant energy as procured from a mercury arc, using full irradiation of varying intensity and using also individual wave-lengths transmitted by a monochromatic illuminator, have been determined. The enzymes investigated are the diastases (amylases) of saliva and of malt and the production, first of erythrodextrin and second, of maltose from starch, is used as an indicator of the enzyme activity. These two phases are designated the dextrinogenic and the saccharogenic. Full irradiation retards the dextrinogenic and the saccharogenic activity of both salivary and malt diastase in an inverse relation to intensity. In the case of salivary diastase the rates of dextrin production and of maltose production are decreased by the green and the far ultra-violet wave-lengths, while both tend toward stimulation when irradiated with the red yellow and near ultra-violet wave-lengths. The monochromatic effects on malt diastase are generally inhibitory for the dextrinogenic phase and stimulatory for the saccharogenic phase. These results may be explained by the presence of two enzymes constituting the diastase, one dextrinogenic, the other saccharogenic; either may be the less active and so become the "pace setter" for maltose production; in the dextrinogenic phase one only is considered, in the saccharogenic phase, both are involved; in salivary diastase the dextrinogenic enzyme is the "pace setter", while in malt diastase the saccharogenic enzyme is usually the "pace setter"; full illumination, however, retards the dextrinogenic enzyme until it becomes the "pace setter". The effects of monochromatic light on the growth of paramecium parallel the effects on the activity of salivary diastase and the effects of monochromatic light on the sporulation of *Colletotrichum* parallel the effects on the saccharogenic activity of malt diastase.

Introduction

There is evidence from results obtained by the authors in their previous work (6, 7, 8, 9), and from the experiments of other investigators, that light has a profound effect on both plant and animal protoplasm and that this effect must be based on one or more basic factors. What these factors are, has yet to be determined. Several theories, however, have been tentatively propounded.

According to F. L. Gates (2) the effect of measured monochromatic ultra-violet energy used to kill bacteria shows characteristic and similar curves at each wave-length, but an appreciable amount of energy must be incident on the bacteria before any of them succumb, and widely different intensities of energy are required to produce these curves at different wave-lengths. The reciprocals of these curves are similar to the absorption curves of certain derivatives of the nucleoproteins which are related to cell growth and reproduction. This conclusion is supported by the known fact that the active agent of chicken tumors is associated with a nucleoprotein, which, in all cases of sarcomatous cells, gives a positive Feulgen reaction. It is therefore possible that the lethal effect and the inhibition of cell division caused by ultra-violet light may be related to its action on the nucleoproteins of the nucleus.

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F. I. Harris and H. S. Hoyt (5) suggest the possibility that the susceptibility of protoplasm to ultra-violet light is conditioned by the selective absorption of the toxic rays by the aromatic amino acid radicals of the proteins.

The authors have suggested (6) that resonance phenomena may be involved, and that certain particles in a colloidal or other state possess vibration periods such that their movements are affected by the specific frequencies of the radiant energy. On this basis subsequent effects may differ from the initial ones owing to associated changes in the colloidal condition.

As there is a certain dependence of enzyme activity on the colloidal state it was thought that the effect of radiant energy on enzyme action might throw some light on the nature of the basic factors causing inhibitory or stimulatory effects on protoplasmic activity.

Review of Literature

Light influences enzyme actions by destroying the enzyme itself or by modifying its activity. Lockemann, Thies and Wichern (11) found that the inhibitory effect of diffused light on peroxidase and blood catalase was in the following order: white, blue, red and darkness. Reinle (13) found that the methylene-blue–formaldehyde–reductase reaction of cow's milk was decreased by the action of ultra-violet light. Getchell and Walton (3) state that ultra-violet light is more potent in the destruction of peroxidase activity than X-rays, radium or visible light.

Pincussen (12) found that the action of light on diastase and urease is dependent on the dilution of the enzyme, the various impurities present and upon the type of the reaction. Radium radiations were also found to be injurious to rennet but to have a stimulative effect on diastase. X-rays also have varied effects on enzymes, the diastatic activity of urine, serum and various fluids being unchanged while that of pepsin and amylase was considerably altered.

H. J. Fuller (1) experimented with plant tissue injured by ultra-violet radiation in respect to catalase and diastase activity and found that the activity of these enzymes actually increased to a considerable extent in tissues exposed to destructive wave-lengths and that there was no evidence whatever of a diminution of enzyme activity, a condition contrary to the results of previous investigators. This author believes that the injurious effects of ultra-violet light must be traceable to some physiological derangement other than the inactivation of enzymes.

It would seem that various forms of radiant energy have varying effects on enzymes from different sources, and the work reported here is an attempt to find some correlation between the effect of light on plant enzymes and on animal enzymes in the hope that some definite organization of the facts may be developed, and that some connection may be established between these results and those previously reported on the effect of ultra-violet light on growth and reproduction.

Experimental

The most widely distributed enzymes that control the hydrolysis of the higher carbohydrates are the diastases. These enzymes hydrolyze carbohydrates to an end-product of achroödextrins and maltose with the intermediate production of erythrodextrins. In these experiments the animal amylase used was ptyalin, the diastatic enzyme present in human saliva. The plant enzyme used was malt diastase from sprouted barley.

The divergence in results obtained during the hydrolysis of starch by diastase of various origins has led to a belief in the presence of two enzymes, a starch liquefying enzyme and a saccharogenic enzyme. In this paper the former is designated as "dextrinogenic". Sherman and Schlesinger (14) and Kendall and Sherman (10) have demonstrated that the liquefying action is predominant in pancreatic amylase and the saccharifying in malt diastase, and have also shown that the liquefying enzyme is predominant in salivary diastase. These workers found that the proportion of the two actions was usually constant for each particular enzyme although great differences in proportion were noticeable in enzymes of varied sources, this difference being ascribed "to the presence of complements or activators which are required for the splitting up of certain definite atomic groups".

It was thought that light, having a specific effect on the action of enzymes, might have a different effect on the two enzymes present in diastase, acting as an inhibitor or activator of the different groups. The experiments here described were therefore carried out on the dextrinogenic action of diastases from various sources as distinct from the saccharogenic action.

A. Salivary Diastase

Materials and Methods

Samples of saliva were obtained in the following manner. The mouth was washed out with distilled water at 40° C.; 20 cc. of water was then held in the mouth for one minute and collected. This was repeated twice. The 40 cc. was then shaken, filtered and the resulting solution kept in the dark. It was found that the activity of the solution became less on the third day, so a fresh solution was made up every three days and was found to be remarkably constant in its activity. The achromic point of the saliva was ascertained and if it varied greatly from four minutes, adjustment was made of the concentration of the solution to obtain an achromic point of constant value.

Soluble starch, c.p., was used as the substrate throughout all the experiments.

Solutions of starch were made up of the following concentrations: 0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5%.

In order to ensure a pH maximum of 6.7, 25 cc. of phosphate buffer of pH 6.7 was used, that pH being the optimum condition for ptyalin activity. Twenty-five cc. of 1% sodium chloride was also added as the action of animal amylase has been proved to be conditioned by the presence of neutral salts in quantities varying from 0.02 to 2.0%.

The 0.1% starch solution would therefore be made up as follows: 5% starch solution, 4 cc.; buffer, 25 cc.; 1% sodium chloride, 25 cc.; distilled water, 146 cc. The other solutions were made up with the required proportions of starch and distilled water.

Procedure

Enzyme solution (3 cc.) was placed in each of two quartz tubes. One tube was fitted into the slit of the monochromatic illuminator or irradiated directly in front of the quartz mercury lamp and the other was kept in the dark as a control.

At the completion of irradiation, 1 cc. of the irradiated solution was diluted to 100 cc. with distilled water and 1 cc. of this solution placed in each of the series of test tubes containing 5 cc. of the graded starch mixtures. Thus 0.01 cc. of the original plus 5 cc. of starch solution was present in each test tube. The same process was repeated for the control. One drop of toluene was added to each test tube, which was then shaken and placed in an oven at 38–40° C. At stated intervals of time one drop was taken from each of the series of test tubes, irradiated and control, placed on a glass plate, using a dropping pipette, and one drop of $N/10$ iodine added. The first appearance of red coloration, denoting conversion of starch to erythrodextrins, was noted and the number of the test tube recorded.

To ascertain the saccharogenic activity of the salivary enzyme, one drop of the irradiated solution was added by means of a dropping pipette to each of two test tubes containing 5 cc. of 4% starch solution. At the same time one drop of the control solution, which had been kept in diffused light in the laboratory, was placed in each of the control test tubes and the entire series placed in the oven at 38–40° C.

After a period of 24 hr. the amount of sugar reduced in the irradiated and control tubes was estimated by means of the Wood-Ost copper carbonate method of sugar estimation (16).

At first readings were taken after six hours' incubation, but the results were found in most cases to be small, the 24-hr. readings giving a more easily recorded result.

Results

1. *The Effect of Full Illumination from a Quartz Mercury Lamp on the Dextrinogenic Action of Saliva*

(*a*) Three cc. of enzyme were irradiated at various distances from a quartz mercury lamp for a period of one hour and the achromic point of the solution ascertained (Table I).

TABLE I

THE EFFECT OF IRRADIATION ON THE SACCHAROGENIC ACTIVITY OF SALIVA

Distance from light source, cm.	30	20	10	7	5	3
Achromic point (time taken to convert starch to achroödextrin and maltose), min.	4	6	12	36	64	No end-point

These results show that the activity of the enzyme is greatly reduced by the action of light in direct relation to the distance from the source.

(*b*) Three-cc. samples of salivary diastase were irradiated for one hour at distances of 10, 7, 5 and 3 cm. from the source of light. The effect of the various irradiated enzyme solutions on the series of starch tubes, as previously described, was ascertained at varying periods of time, and a comparison made with the control (Table II, Fig. 1).

TABLE II

THE EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE DEXTRINOGENIC ACTIVITY OF SALIVARY DIASTASE

(Activity of enzyme expressed as percentage concentration of starch solution hydrolyzed to erythrodextrin in a measured time)

Distance from light source, cm.	Duration of digestion in hours							
	1	2	3	6	10	24	48	96
Control	0.5	1.0	1.5	2.0	2.5	3.0	4.0	5.0
10	.5	.75	1.0	1.5	2.0	2.5	3.0	4.0
7	.2	—	.5	1.0	1.5	2.0	2.5	3.0
5	.2	—	.3	.5	.75	1.0	1.5	2.0
3	.1	—	—	.2	—	—	—	—

It will be seen that the full light from a mercury arc lamp has a decided inhibitory action on the activity of salivary diastase, evidently in varying proportion to the intensity of the light and the time. The rise in temperature of the extracts so irradiated was negligible as compared with the control, except in the case of irradiation at a distance of 3 cm. from the source of light, when the temperature was raised about 3° C. above room temperature. The almost total inactivation of the enzyme so irradiated may be therefore partly due to a possible destructive action of the wave-lengths of the infra-red part of the spectrum. Similar results were obtained when the saccharogenic activity was measured after an identical series of irradiations.

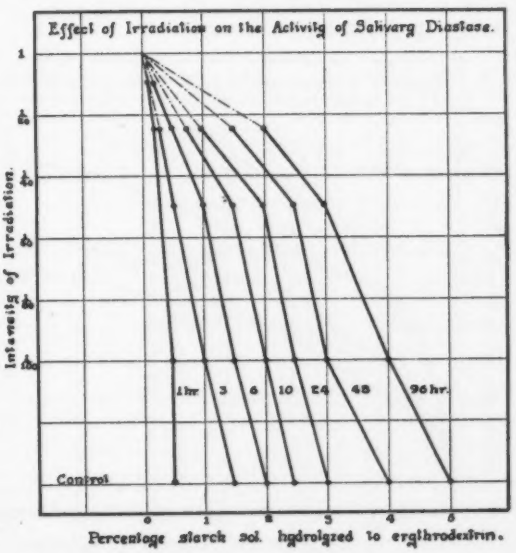

FIG. 1. *The effect of radiant energy of various intensities upon the rate of salivary diastase activity, as measured by the erythrodextrin produced.*

The experiments to determine the effect of full irradiation at different distances, as compared with a control kept in room light, show that the magnitude of the effect varies with the intensity, under these conditions. The curves (Fig. 1) representing the percentage of starch hydrolyzed to erythrodextrin after exposure to various intensities, regulated by distance from the light source, show that the inhibiting effect increases at a greater rate than the reciprocal of the square of the distance. The intensity of the light at the distances indicated has not been determined directly but it is presumed that in this case the intensity varies as the reciprocal of the square of the distance. It would appear that the inhibiting effect is proportionately greater at higher intensities. The marked similarity of the curves representing the percentages of starch hydrolyzed after irradiation during the time periods, 1, 3, 6, 10, 24, 48 and 96 hr. indicates that the effect of irradiation upon the enzyme endures and is constant for a period of four days after the experimental exposures to radiant energy.

The Specific Effect of Monochromatic Light on the Dextrinogenic and Saccharogenic Activities of Salivary Diastase (Tables III and IV. Figs. 2, 3 and 4)

Class 1. Wave-lengths having no dextrinogenic effect. The wave-lengths λ 6152 Å, λ 5819 Å and λ 3821 Å seem to exert no appreciable effect on the dextrinogenic action of the diastase used, whereas λ 6152 Å and λ 3821 Å exert a minimal stimulating effect, and λ 5819 Å a greater stimulating effect, on the saccharogenic activity of the diastase.

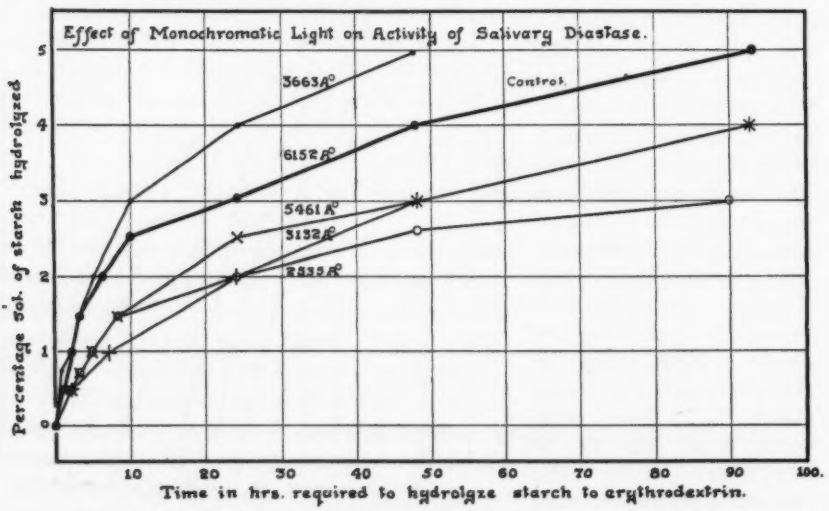

FIG. 2. *The effect of specific light wave-lengths of the mercury arc spectrum on the dextrinogenic activity of salivary diastase.*

Class 2. Wave-lengths having a stimulating effect. λ 3663 Å is apparently the only line exerting stimulation on both saccharogenic and dextrinogenic activities, whereas λ 6152 Å, λ 5819 Å, λ5461 Å and λ 3821 Å stimulate the

sugar forming portion of the diastase and λ 4359 Å and λ 4078 Å give a slight initial stimulation of the dextrin producing portion.

TABLE III

THE SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE DEXTRINOGENIC ACTIVITY OF SALIVARY DIASTASE

(Activity of the enzyme expressed as percentage concentration of starch solution hydrolyzed to erythrodextrin in a given time. Enzyme irradiated for 24 hours)

Wave-length	Hours of digestion													Total*	Diff.**
	1	2	3	4	5	6	7	8	9	10	24	48	96		
Control	.5	1.0	1.5	–	–	2.0	–	–	–	2.5	3.0	4.0	5.0	15.0	–
6152 Å	.5	1.0	1.5	–	–	2.0	–	–	–	2.5	3.0	4.0	5.0	15.0	0.0
5819 Å	.5	1.0	1.5	–	–	2.0	–	–	–	2.5	3.0	4.0	5.0	15.0	0.0
5461 Å	.3	.5	.75	–	1.0	–	–	1.5	–	–	2.5	3.0	4.0	11.05	−3.95
4916 Å	.3	.5	.75	1.0	–	1.5	–	–	–	2.0	2.5	3.0	4.0	11.05	−3.95
4359 Å	.75	1.0	1.5	–	2.0	–	–	–	–	2.0	3.0	4.0	5.0	15.25	0.25
4078 Å	.75	1.0	1.5	–	–	2.0	–	–	–	2.0	3.0	4.0	5.0	15.25	0.25
3821 Å	.5	1.0	1.5	–	–	2.0	–	–	–	2.5	3.0	4.0	5.0	15.00	0.0
3663 Å	.5	1.0	1.5	–	2.0	–	–	–	–	3.0	4.0	5.0	5.0	17.00	2.0
3132 Å	.3	.3	.5	–	.75	–	1.0	–	–	–	2.0	3.0	4.0	10.10	−4.9
3022 Å	.3	.5	.75	–	–	1.0	–	–	–	–	3.0	4.0	4.0	12.55	−2.45
2967 Å	.3	.5	.75	–	–	1.0	–	–	–	–	2.0	3.0	4.0	10.55	−4.45
2804 Å	.3	.5	.75	–	–	–	1.0	–	–	–	2.5	3.0	4.0	11.05	−3.95
2700 Å	.5	.5	.75	–	–	1.0	–	–	–	–	2.0	3.0	4.0	10.75	−4.25
2535 Å	.3	.5	.75	–	–	1.0	–	–	1.5	–	2.0	2.5	3.0	9.05	−5.95

**Total—Sum of data for 1, 2, 3, 24, 48 and 96 hr.*

***Diff.—Difference between the activity of the control and of the irradiated enzyme.*

TABLE IV

EFFECT OF MONOCHROMATIC LIGHT ON THE SACCHAROGENIC ACTIVITY OF SALIVARY DIASTASE

(Readings expressed as mgm. maltose present after 24 hours' digestion)

Wave-length	Control	Irradiated	% Difference	Mean effect
6152 Å	50	50	0	2.4
	64	67	4.8	
5819 Å	58	65	13.8	12.12
	62.5	69	10.5	
5461 Å	57	60	2.5	3.85
	59	60.5	5.2	
4916 Å	52	42.5	18.2	−19.5
	57.5	45.5	20.8	
4359 Å	56	50	10.7	−10.6
	55	49	10.9	
4078 Å	50	35	30.0	−27.2
	49	37	24.4	
3821 Å	42.5	44	3.5	3.5
3663 Å	53	55	3.7	5.1
	45	48	6.6	
3132 Å	61	55	9.8	−11.2
	55	48	12.7	
3022 Å	69	55	16.8	−15.2
	66	58	13.6	
2967 Å	52	45	13.4	−19.2
	56	42	25.0	
2804 Å	53	42	17.0	−19.3
	57.5	45	21.7	

Class 3. Wave-lengths exerting an inhibitory effect. λ 5461 Å, λ 4359 Å, λ 4078 Å and the ultra-violet mercury lines used, λ 3132 Å, λ 3022 Å, λ 2967 Å and λ 2800 Å, caused a decided inhibition of the dextrinogenic activity. Of these, λ 5461 Å gives a slight saccharogenic stimulation. In no case is the activity entirely destroyed by monochromatic light of the low intensity used.

General

It would seem from these results (Fig. 3) that the specific effects of the monochromatic light of the far ultra-violet spectrum (*i.e.*, beyond λ 3663 Å) are very similar for all phases of diastatic action in saliva, and that these

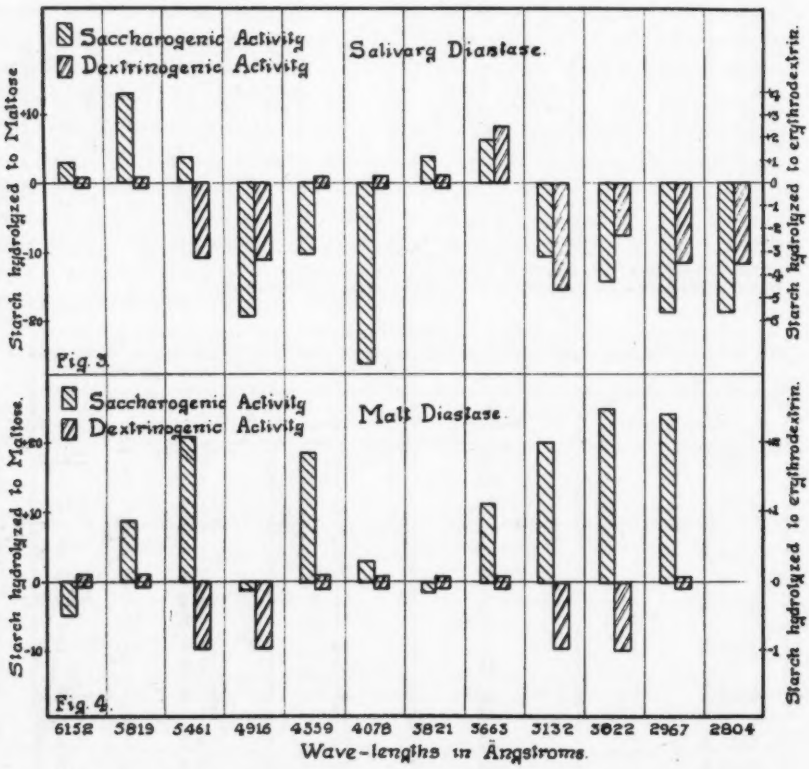

FIG. 3. *A graph to compare the specific effects of various light wave-lengths on the dextrinogenic and the saccharogenic phases of hydrolysis by salivary diastase.*

FIG. 4. *A graph to compare the specific effects of various light wave-lengths on the dextrinogenic and the saccharogenic phases of hydrolysis by malt diastase.*

effects are decidedly inhibitory and cause a reduction in the rate of the reaction. The near ultra-violet (λ 3821 Å, λ 3663 Å) spectrum seems to cause a slight stimulation which changes to decided inhibition for all phases as the green portion of the spectrum is approached. Stimulation again characterizes

the yellow-red end of the visible spectrum; λ 5461 Å, being transitional, is the only line showing contrary effects for dextrinogenic and saccharogenic activity. It would seem, however, that the saccharogenic portion of the salivary diastase is more readily affected by irradiation, stimulation and inhibition being reported for lines of the spectrum which cause slight or undetermined effects on the dextrinogenic portion of the enzyme.

The Time Factor in Relation to the Light Effect on Diastase

The time-hydrolysis curve of the control (Fig. 2) illustrates the relatively rapid rate of digestion during the early period and the decreasing rate as the time increases. At 3 hr. the 1.5% starch solution is hydrolyzed to erythrodextrin; at 10 hr. the 2.5% solution is hydrolyzed and at 96 hr. the 5.0%. Other experiments (15) have shown that an optimum concentration of substrate characterizes such reactions, but the results cannot be explained on this basis unless the optimum is below 0·3% starch solution. Generally the curves showing either retardation (λ 5461 Å and λ 2535 Å) or stimulation (λ 3663 Å) show a type of time-rate curve similar to that of the control. On the contrary, λ 3132 Å causes a primary retardation which is relatively greater, while λ 4359 Å and λ 4078 Å cause a primary stimulation for one hour followed by an absence of appreciable effect. Again, it would appear that with slight exception the modification of the enzyme induced by irradiation persists during the four-day period of its subsequent activity.

B. Malt Diastase

Malt diastase (0.75 gm.) was shaken for 15 min. in 250 cc. of distilled water and then filtered. This solution, plus two drops of toluene as a preservative, was kept in the dark in a stoppered bottle.

The same concentrations of starch solutions were made up as were used for the salivary series, but the pH was kept between 4.8 and 5.2 by means of a phthalate buffer. The 0.1% starch solutions were therefore made up as follows:—5% starch solution, 4 cc.; buffer, 25 cc.; distilled water, 171 cc.

Enzyme solutions (3 cc.) were placed in each of two quartz tubes and irradiated as in the previous experiment.

To ascertain the dextrinogenic action of the diastase three drops of the irradiated malt diastase was added by means of a dropping pipette to the starch series with two drops of toluene per test tube, shaken, and placed in the oven at 38–40° C. Three drops of the non-irradiated control was also added to another series and that in turn placed in the oven.

A record was made of the test tube in which the first appearance of red coloration, denoting conversion of starch to erythrodextrin, was noted.

To ascertain the saccharogenic activity of the enzyme the same procedure was followed as for salivary diastase, except that the amount of sugar reduced was ascertained after only six hours' incubation, the saccharogenic action in this case being more rapid than is that of the salivary diastase.

The amount of maltose produced was measured by means of the Wood-Ost copper carbonate method of sugar estimation (16).

Results

1. Effect of Full Illumination from a Quartz Mercury Lamp on the Activity of Malt Diastase

A decided inhibitory effect was recorded, both for the dextrinogenic and sugar-reducing activities, increasing with the intensity of the light, the greatest inhibition being found when the solutions were irradiated at 3 cm. distance from the source of light (Tables V and VI).

TABLE V

EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE DEXTRINOGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Distance from source, cm.	Days of digestion											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.5	2.0	–	2.5	–	–	3.0	–	–	4.0	–	–
10	1.0	1.5	–	–	–	2.5	–	–	–	3.0	–	–
7	1.0	1.5	–	–	2.0	–	–	–	2.5	3.0	–	–
5	.75	1.0	–	–	1.5	–	–	–	–	2.5	–	–
3	.3	.5	–	.75	–	1.0	–	–	–	1.5	–	–

TABLE VI

EFFECT OF FULL ILLUMINATION FROM A QUARTZ MERCURY LAMP ON THE SACCHAROGENIC ACTIVITY OF MALT DIASTASE

(Readings expressed as mgm. maltose present after 6 hours' digestion)

Distance from source, cm.	Exp.	%	Exp.	%	Exp.	%	Av. %
Control	12.5	100	31	100	22	100	100
10	6.0	48	16	51	10	45	46
5	5.5	44	12	39	8.5	40	41
3	4.0	32	8	26	7.0	32	30

TABLE VII

SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE DEXTRINOGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Wave-length	Days of digestion											
	1	2	3	4	5	6	7	8	9	10	11	12
Control	1.5	2.0	–	2.5	–	–	3.0	–	–	4.0	–	–
5461 Å	1.5	–	–	2.0	–	–	2.5	–	–	3.0	–	–
4916 Å	1.5	–	–	–	2.0	–	2.5	–	–	3.0	–	4.0
3132 Å	1.5	–	2.0	–	–	–	2.5	–	–	3.0	–	4.0
3022 Å	1.5	–	–	2.0	–	–	–	2.5	–	3.0	–	4.0

NOTE:—*Wave-lengths 6152 Å, 5819 Å, 4359 Å, 4078 Å, 3821 Å, 3663 Å, 2967 Å, 2804 Å, 2700 Å, 2535 A showed no effect (same as control).*

2. The Specific Effects of Monochromatic Light on:—

A. The dextrinogenic activity of malt diastase (Table VII). It was found that only four wave-lengths, *i.e.*, λ 5461 Å, λ 4916 Å, λ 3132 Å and λ 3022 Å, had any appreciable effect on the diastatic action of the malt solution. The lack of effect in the shorter wave-lengths may be due largely to the fact that the intensity of the incident light was not sufficient to initiate any change in enzyme action, although it must be noted that λ 4916 Å, λ 3132 Å and λ 3022 Å are all lines of very low intensity, as compared with λ 5461 Å, and all exert a decidedly inhibitory action, pointing to the fact that intensity is not the controlling factor in this case.

B. The saccharogenic activity of malt diastase (Table VIII). λ 6152 Å, λ 4916 Å and λ 3821 Å all exert a slight inhibitory action, whereas all other lines used, whether in the visible or ultra-violet spectrum, exert a decided stimulation, in some cases exerting stimulation identical with that caused by the same lines on the dextrinogenic action and in other cases being the direct opposite.

TABLE VIII

THE SPECIFIC EFFECTS OF MONOCHROMATIC LIGHT ON THE SACCHAROGENIC ACTIVITY OF MALT DIASTASE

(Enzyme activity expressed as percentage concentration of starch solution completely hydrolyzed in a given time. Readings after 24 hours' irradiation)

Wave-length	Irradiated	Control	% Diff.	Average effect
6152 Å	15.5	16.0	3.2	
	14.0	15.0	7.1	−5.15
5819 Å	10.0	9.0	10.0	
	12.5	11.5	8.0	9.0
5461 Å	37.0	29.0	21.6	
	25.0	19.0	26.9	
	20.0	17.0	15.0	21.7
4916 Å	24.0	24.0	0	
	25.0	24.5	2.0	1.0
4359 Å	16.0	12.0	25.0	
	18.0	14.0	22.2	
	35.0	31.5	10.0	19.1
4078 Å	24.0	24.0	0	
	21.0	20.0	5.0	
	15.0	14.5	3.3	2.7
3821 Å	27.0	27.0	0	
	15.0	15.5	3.0	−1.5
3663 Å	17.0	16.0	6.0	
	18.0	15.0	16.5	11.2
3132 Å	36.0	28.0	22.2	
	38.0	30.0	21.2	21.7
3022 Å	18.0	16.0	11.1	
	26.0	17.0	34.5	
	28.0	18.0	35.7	
	16.0	12.0	25.0	26.6
2967 Å	19.0	14.0	26.3	
	21.0	16.0	23.8	25.0
2804 Å	17.0	17.0	0	0
2700 Å	19.0	19.5	0	.5

These results are a little difficult to explain, owing to the fact that illumination from the full mercury spectrum caused decided inhibition in saccharogenic activity. These results, however, may have some bearing on those of H. J. Fuller, who reports that the activity of catalase and plant diastase is increased to a considerable extent in tissues exposed to otherwise inhibitory wave-lengths, and found no diminution of enzyme activity. A possible explanation is offered later under the heading of "pace setter".

In comparing these two sets of results there is evidence that, while the lines of the mercury arc spectrum which exert a measurable effect on the dextrinogenic activity of the malt diastase, cause inhibition, the effect of the monochromatic lines, except those of the red end of the visible spectrum, on the saccharogenic activity is stimulatory.

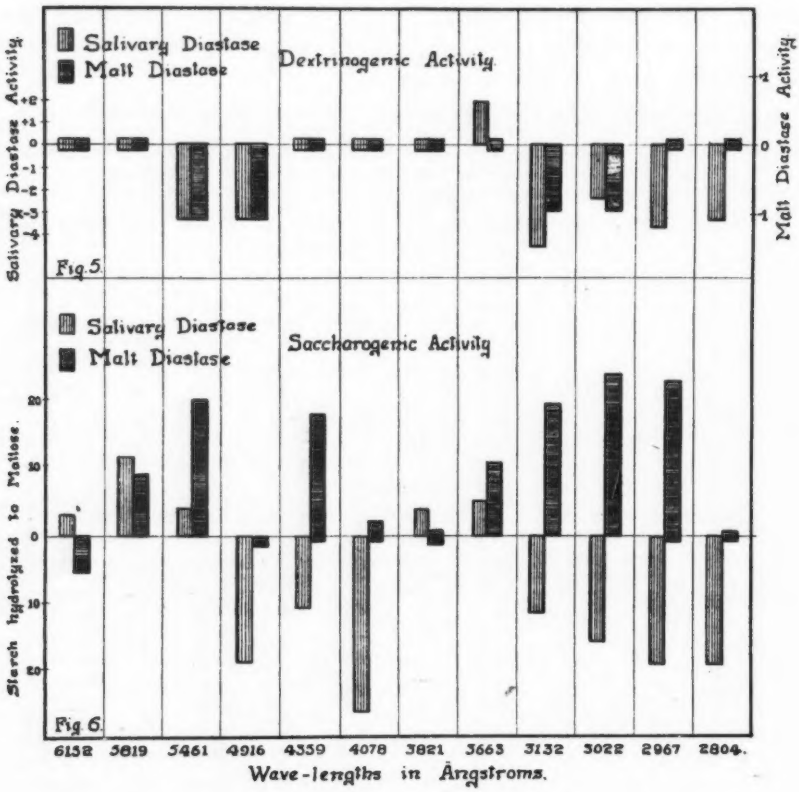

FIG. 5. *A graph to compare the specific effects of various light wave-lengths on the dextrinogenic activity of salivary and of malt diastase.*

FIG. 6. *A graph to compare the specific effects of various light wave-lengths on the saccharogenic activity of salivary and of malt diastase.*

Comparison of the Effects of Irradiation on the Dextrinogenic Activity of the Two Diastases (Fig. 5)

In these experiments the salivary diastase is most affected by the irradiation used, there being two portions of the spectrum, that around the green region of the visible spectrum, and that in the far ultra-violet region, which cause distinct inhibition of action. One line only, λ 3663 Å, exerts a stimulating effect. The malt diastase is at no time stimulated, and although inhibited by the same wave-lengths of the green region as is salivary diastase, inhibition is confined elsewhere to two lines, λ 3122 Å and λ 3022 Å; the far ultra-violet apparently having no appreciable effect in this case.

Comparison of the Effect of Irradiation on the Saccharogenic Activity of the Two Diastases (Fig. 6)

In the case of the salivary diastase the red-yellow and the near ultra-violet portions of the spectrum exert stimulation, but the region of the green-violet lines and the far ultra-violet exert decided inhibition of enzyme action, the visible lines giving an even greater effect than the ultra-violet lines. On the other hand, with the exception of λ 6152 Å, λ 4916 Å and λ 3821 Å, which exert an almost negligible negative effect, the malt diastase activity is greatly stimulated, not only by the lines of the visible spectrum but also by the ultra-violet lines of intermediate wave-length. The only wave-lengths which have like effects on the malt and salivary saccharogenic activity are the orange, yellow and near ultra-violet.

Comparison of the General Effect of Irradiation on the Two Diastases

It would seem, in the experiments here reported, that the effect of irradiation is greater in the case of the saccharogenic activity of both enzymes, and that the dextrinogenic activity is affected to a lesser extent.

It is also of interest to note that salivary diastase, on the whole, is affected to a greater extent than malt diastase, and that the inhibitory effect predominates. In malt diastase, the dextrinogenic activity is inhibited by only four lines, and the saccharogenic activity is generally stimulated.

The Diastatic "Pace Setter"

The production of erythrodextrin is dependent upon the activity of the dextrinogenic enzyme phase, while that of maltose is dependent on the two phases, dextrinogenic and saccharogenic. However, since the slowest action in any series is the "pace setter", and since the effects of ultra-violet light on the dextrinogenic action is similar in the two cases, these experiments indicate that the dextrinogenic activity is the "pace setter" for salivary diastase while the saccharogenic activity is the "pace setter" for malt diastase. This result seems to agree with the conclusions of Sherman, Schlesinger and Kendall (10, 14), that the liquefying action is predominant in salivary diastase, while the saccharifying action is predominant in malt diastase. The parallelism between the measured effects on the dextrinogenic phases of hydrolyses for salivary and malt diastase indicates that the dextrinogenic phases are similar in both

enzymes. It is possible that the effect on the saccharogenic enzyme phases may be the same in each also, but in the case of the salivary diastase the effect is masked by the controlling dextrinogenic phenomena. The saccharogenic effect on malt diastase is controlled by the dextrinogenic phase only when the intensity of irradiation is sufficiently great to constitute the latter as "pace setter", as in the case of full irradiation.

Evidence for Two Enzymes

The differences in the effects of monochromatic light upon the saccharogenic activity of malt diastase as compared with salivary diastase, in contrast with the similarities of the dextrinogenic effects together with the inversion of the effect of monochromatic light as compared with that of full irradiation on the saccharogenic activity of malt diastase, indicate that the two phases of starch digestion are each dependent upon a separate enzyme or that the enzyme changes its light relations. The results are more readily explained by postulating two enzymes, one dextrinogenic, and the other saccharogenic. Under ordinary conditions, as in malt diastase, the saccharogenic may be the "pace setter". Light may retard the activity of the dextrinogenic to such an extent that it becomes the "pace setter". This evidence is in accord with the conclusions of Sherman, Schlesinger, Kendall and others on the nature of the enzymes involved in the hydrolysis of starch (10, 14).

A Comparison of the Effect of Monochromatic Light on the Growth of Paramecium, and on the Diastatic Activity of Saliva (Fig. 7)

The very close parallelism is illustrated by Fig. 7. Of the wave-lengths tested, two only show effects differing in direction and these (λ 5461 Å and λ 3821 Å) are in transition regions, between regions of stimulatory and inhibi-

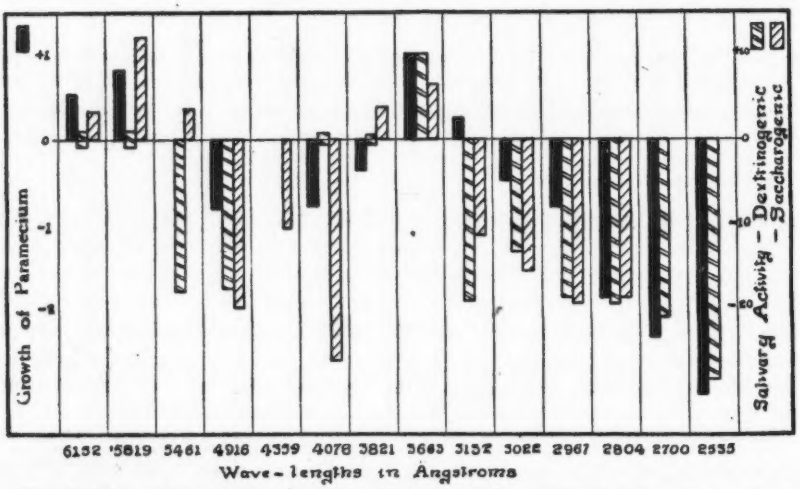

FIG. 7. *A graph to compare the effects of various light wave-lengths upon the growth of paramecium and the activity of salivary diastase, dextrinogenic and saccharogenic.*

tory effects. For other wave-lengths not only is stimulation or retardation of growth associated with stimulation or retardation of the enzyme activity respectively, but, in most instances, the magnitudes of the two effects are relatively in the same order. This parallelism is even more remarkable since the enzyme is that of man while the rate of growth is that of a unicellular organism. At present data are not available for the diastase of paramecium. The parallelism suggests that the growth of certain organisms may be limited by the food made available by enzymes such as diastase.

A Comparison of the Effect of Monochromatic Light on the Sporulation of Colletotrichum and on the Saccharogenic Activity of Malt Diastase

Of the several phenomena studied by the authors (6, 7, 8, 9), including the effect on growth of several organisms (6, 7, 9), the effect on plasmolysis (8), the effect on sporulation (7) and on enzyme activity, only two exhibit

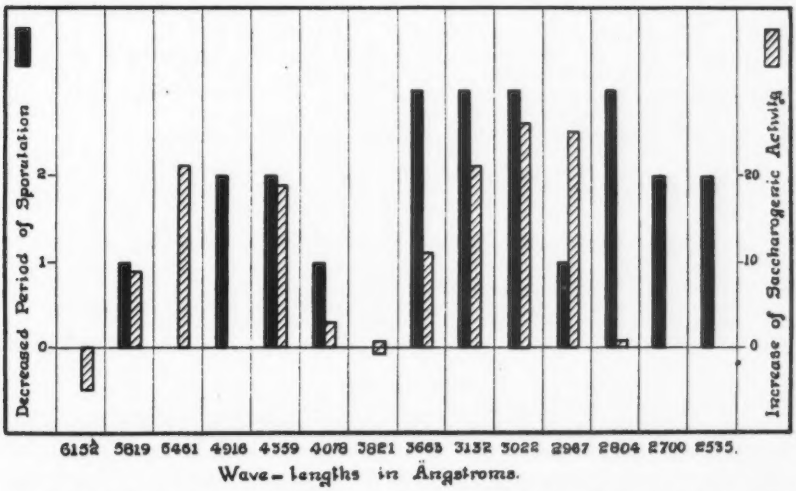

FIG. 8. *A graph to compare the effects of various light wave-lengths upon the sporulation of Colletotrichum (Tomato rot) and the saccharogenic activity of malt diastase.*

a consistent positive response to the several wave-lengths of monochromatic light, namely, sporulation in *Colletotrichum* and the saccharogenic activity of malt diastase. The parallelism is shown in Fig. 8. Spores frequently store abundant sugar and this is probably true of *Colletotrichum*. The parallelism suggests that the formation of spores may be limited by the production of maltose as regulated by saccharogenic enzymes.

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AN INVESTIGATION OF THE SURFACE TENSION OF LIQUIDS NEAR THE CRITICAL TEMPERATURE[1]

By C. A. Winkler[2] and O. Maass[3]

Abstract

The surface tension-temperature relations of methyl ether and propylene have been investigated by the capillary rise method over a range of temperatures approaching more closely to the critical temperature than has hitherto been done. From the data obtained, it has been concluded that the molecular surface energy does not become equal to zero when the meniscus is no longer visible, since the surface tension-temperature curves obtained apparently did not become asymptotic to the temperature axis at the critical temperature, the latter being considered as the temperature at which the meniscus is no longer discernible by the wave-lengths of visible light. The angle of interception of the surface tension-temperature curve with the temperature axis has been interpreted as indicating a discontinuity in properties at the critical point.

The data obtained have also been applied to the examination of various relations involving surface tension. The Katayama equation has been shown to be considerably more accurate than that of Ramsay and Shields. Sugden's equation, has been found valid over the ranges of temperature investigated. The Macleod relation has also been examined, and an increase in the Macleod constant with increase in temperature shown to occur in the case of propylene, while no marked or progressive increase was noticed in the case of methyl ether. Calculation of the parachors served to emphasize the difference in behavior of the two substances in this respect. By a consideration of available data on benzene, chlorobenzene, and carbon tetrachloride, together with those obtained in this investigation, the increase of parachor in the case of those substances having unsaturated linkages in their molecular structure has been ascribed to increased electronic interaction with increased temperature. This is analogous to an increase in unsaturation.

An attempt was made to adapt the ring method to the investigation of surface tension in the critical region, by measuring the force necessary to effect removal of the ring from the surface of the liquid by means of a calibrated quartz spiral. Although the method was unsatisfactory for the problem at hand, the feasibility of the method has been demonstrated. From the data obtained it has been possible to verify the zero angle of contact between methyl ether and glass.

Introduction

The accurate determination of the surface tension of liquids has received the attention of numerous workers. The rise of liquids in capillary tubes was first studied by Leonardo da Vinci, in the seventeenth century, but it was not until von Segner (17) introduced the conception of surface tension that the subject became of fundamental interest and importance. The first correct theory of the rise of liquids in capillary tubes was put forward by Leslie (8) in 1802, but the mathematical treatment was not expounded until Young (26), Laplace (7), and others gave it their consideration. Since that time, the theory of surface tension has received considerable modification and development. Paralleling the theoretical development, the experimental technique for determining the surface tension of a liquid has also advanced to a remarkable extent. New methods have been devised,

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and older methods modified; according to Ferguson (5) some 20 methods have been investigated, of which the capillary rise method and the ring method are probably most commonly used at the present time.

The temperature coefficient of surface tension has also received considerable attention. In general, it is not large (about 0.5% per degree), and is negative. Over short temperature ranges, the surface tension-temperature relation is practically linear, but observations over extended ranges of temperature (13) show that the surface tension-temperature graph exhibits a decided curvature. Furthermore, a rapid diminution in the slope of the curve near the critical temperature was observed.

A careful review of the published data reveals that, although the temperature coefficients of surface tension for numerous liquids are well established for considerable temperature ranges, surprisingly few investigations have been extended to the critical point. In all, probably not more than six liquids have been studied at temperatures approaching the critical, and even for these the investigations have not included temperatures closer than approximately 5°C. to the critical temperature. Graphical representation of the published data for these substances indicates that the surface tension diminishes to zero value at the critical point.

Recent investigations in this laboratory suggested the possibility, however, that partial orientation of the molecules at the liquid-vapor interface is maintained even after the critical temperature is attained. Should this be the case, the surface tension-temperature curve might be expected to extrapolate to zero value of the surface tension at some temperature above the critical. Although purely speculative in its origin, this problem demanded experimental investigation, since the data available were not sufficient to provide the solution. Consequently, it was decided to determine the surface tension of some liquids over as large a temperature range as possible, in an endeavor to establish the surface tension-temperature relation closer to the critical temperature than had previously been done.

Experimental

Of the numerous methods for the determination of surface tension, the ring and the capillary rise methods were deemed most readily applicable in the critical region. The former proved to be somewhat cumbersome, however, and was rejected in favor of the capillary rise method. A brief description of the ring method will be given, since the manner in which it was applied to the present problem, and the results obtained thereby, might prove of considerable interest.

(*a*) *The Ring Method*

This method, initiated by Sondhaus (18), and developed to some extent by Timberg (23) and Weinberg (24), did not become generally applicable to the determination of surface tension until Du Noüy (3, 4) devised the tensiometer which bears his name. The principle employed by Du Noüy did not

seem applicable, however, in a system which must be closed, under a pressure of some 50 atmospheres, at a temperature of approximately 125°C. Consequently, a quartz spiral replaced the torsion balance for measuring the force required to effect removal of the ring from the surface of the liquid.

A platinum ring, of approximately 1 cm. circumference, was suspended from a machine-wound, calibrated quartz spiral, the sensitivity of which was 0.00194 gm. per mm. extension. For details concerning the method of winding such spirals, the reader is referred to a paper by Tapp (22), who very kindly furnished the spiral used in this investigation.

FIG. 1. *Diagram of the apparatus for the ring method.*

The necessity for obtaining a uniform upward motion of the ring through the surface of the liquid presented a problem of very great difficulty; indeed, the ring method was finally abandoned for this reason. Many devices, which promised successful operation, had to be abandoned in actual practice. The apparatus finally employed was not all that was desired from the standpoint of manipulative ease, but did indicate the feasibility of the method, and gave promise of the development of a satisfactory technique for the determination of the surface tension of a liquid, near the critical temperature, by the ring method.

The spiral and attached ring were suspended by a silk thread from a glass sheath, which enclosed a small iron nail. The thread was of a length calculated to satisfy the required elevation of the ring, as determined by the dimensions of the complete assembly. At each end of the sheath, symmetrically about it, were sealed three fine glass rods, at right angles to the axis of the nail. The lengths of these rods were such that they did not quite touch the inner wall of the glass bomb which was to contain the mechanism. In this manner, the sheath around the nail was held centrally in the bomb, and made contact with the wall at only four points at any time.

The bomb which contained the spiral assembly was of Pyrex tubing, with an internal diameter of 1.5 cm., and a wall thickness of 4 mm. After sealing off one end of the tube, indentations were made in the wall at an appropriate distance from the bottom, or sealed end, these indentations being diametrically opposite. A short piece of tubing, inserted into the bomb and allowed to rest on the indentations, served to support the spiral assembly while the apparatus was not in use, since, upon lowering the assembly, the short projecting rods on the sheath came to rest on the upper edge of this short tube. At a calculated distance above the sheath, the bomb was drawn down, and a short piece of capillary tubing sealed to it, through which the liquid could be introduced.

Methyl ether was introduced by low temperature distillation, until the liquid occupied $\frac{2}{3}\frac{4}{3}$ of the total bomb space, at -78.5°C. This ratio of liquid to total bomb space was maintained to minimize the motion of the meniscus near the critical temperature. The bomb was then sealed off, and immersed

in a bath of glycoline oil. The bottom of the bomb rested on a cork support; to the top was fixed a collar supported on one end of a long brass rod, which extended some distance from the bath. At the other end of this rod was fixed a double system of racks and pinions, which enabled the operator, at a distance from the bath, to move the top of the bomb in any direction. This enabled the operator to keep the ring in the centre of the liquid surface, a necessary feature, since there was a slight tendency for the ring to draw towards the wall of the bomb at the instant of release.

To further decrease the tendency for the glass sheath surrounding the nail to bear lightly against the wall of the bomb, and thus impair the production of an even force on the ring, a very rapid, but very slight, vibration was imparted to the upper portion of the bomb. This was done by permitting the core of a small solenoid, through which an alternating current was passing, to bear very lightly against the bomb. The amount of vibration could be regulated and was never permitted to increase to an extent which would cause small ripples on the surface of the liquid in the lower portion of the bomb.

The spiral was raised by means of a small solenoid placed around the bomb. In this way, the force tending to draw the nail over to the wall in one direction was never greatly in excess of that tending to draw it in another, a factor which aided in the attainment of a steady pull on the ring. This solenoid could be raised and lowered by the operator at some distance from the bath.

The temperature of the bath was controlled manually, and ascertained on a standard thermometer, with an error not exceeding 0.03°C. The extension of the spiral was determined with a cathetometer reading to 0.02 mm. In observing this extension, it was necessary to follow the upper extremity of the spiral, as it was being raised, with the cathetometer, the reading at the instant that the ring broke from the surface being noted. The release of the ring from the surface was accompanied by a slight, but detectable, jerk in the upward motion of the spiral. In any case where this was not noticed, the reading was not recorded. At the higher temperatures, the release was not as noticeable as at the lower temperatures, but with care could be fairly readily detected. The reading at the point at which the meniscus stood was taken in each case, and the difference between this reading and that of the upper extremity of the spiral, minus the normal length of the spiral with ring attached, gave the extension produced in the spiral due to the force necessary to release the ring from the surface of the liquid. Since the spiral was calibrated, the only factor remaining unknown was the circumference of the ring. This was determined in a manner to be indicated later, after which the surface tension of methyl ether was readily calculable.

(*b*) *The Capillary Rise Method*

The capillary rise method was finally adopted for this investigation for two main reasons. A high degree of accuracy was of fundamental importance, since a small surface tension prevails in the region it was desired to investi-

gate. The capillary rise method answers this requirement, being probably the most accurate method yet devised. Furthermore, it soon became evident that the capillary rise method should be more readily applicable under the experimental conditions encountered than the ring method first attempted.

While it is not the intention to review in detail the very extensive literature dealing with the determination of surface tension by the rise in a capillary tube, some of the important considerations indicated by the work of others might be of interest. Richards and his coworkers (14, 15, 16) have demonstrated the necessity for scrupulous cleanliness, uniformity and non-ellipticity of the bore of the capillary tube, smoothness of its internal surface, and good optical properties of the capillary walls. They have also indicated the advantages and disadvantages of various arrangements of the smaller and larger tubes of the capillarimeter. Predescu (12) suggested a modification in the construction and manipulation of the apparatus which reduces the error due to variations in the bore of the capillary tube. Sugden (19) described a modified capillarimeter designed to obviate difficulties arising from the absence of a plane surface of reference.

In preliminary work, the smaller capillary was sealed at both its ends externally into the wall of a larger capillary tube, a design which is desirable from the standpoint of accuracy. The technical difficulties encountered in constructing such a capillarimeter proved to be very great. Although it was possible, after a number of attempts, to obtain sufficient mechanical strength to withstand the pressures imposed, it was not found possible to control the temperature of the apparatus sufficiently well with the facilities available. While fluctuations in the temperature of the bath as a whole, as recorded thermometrically, could be reduced to the desired extent, slight regional fluctuations could not be eliminated. Distillation of the liquid from one portion to another of the smaller capillary as a result of these regional fluctuations in temperature necessitated the abandonment of this type of capillarimeter.

The second type of apparatus used proved successful. In this type, the small capillary was contained inside a heavy-walled Pyrex glass bomb. Since the external diameter of the capillary was approximately 1 mm. as compared with an internal diameter of 15 mm. of the larger tube, the surface of the liquid was practically plane, especially at the higher temperatures. Consequently, the degree of accuracy was estimated to be not greatly inferior to that attainable with the first type of capillarimeter.

Calculation showed the necessity for a capillary of approximately 0.1 mm. internal diameter, to permit of sufficient accuracy. One satisfying this requirement, and also the other specifications mentioned, was selected.

After cleaning successively with chromic acid, distilled water, alcohol and ether, and drying carefully, the capillary was inserted into a short piece of capillary tubing of the proper bore to hold it rigidly. This supporting capillary was chosen of such an external diameter that it fitted tightly into the larger bomb tubing.

The bomb was of Pyrex glass, with a wall thickness of 4 mm., and an internal diameter of 15 mm. It was selected with special reference to its optical properties. After thorough cleaning and drying, one end was sealed off, and two indentations made in the wall at diametrically opposite points. The capillary tube, mounted as previously described, was then inserted, and forced down until the supporting short piece of capillary came to rest on the indentations in the wall of the bomb. Care was taken to ensure a vertical position of the capillary. The bomb was then sealed to a low temperature fractionating unit, and the desired liquid introduced by distillation.

Methyl ether was prepared and purified as described by Winkler and Maass (25). Propylene was prepared and purified to constant vapor pressure as outlined by Coffin and Maass (2) and Maass and Wright (10). The pure gas (either methyl ether or propylene) was condensed into the bomb by surrounding it with a freezing mixture of solid carbon dioxide and acetone, after which it was sealed off.

The temperature was controlled by immersing the bomb in a bath of electrically heated glycoline oil. A thermoregulator was provided to maintain the temperature constant to within 0.2°C. of the desired temperature. Closer regulation was effected by manual control of the rheostats connected in series with the heating element, as will be described later. The temperature was ascertained on a standardized thermometer, with an error not exceeding 0.03°C. Owing to the possibility of the bomb shattering at the higher temperatures, the operator was protected by a housing which enclosed the apparatus, a non-shatterable glass window being provided through which the observations could be made. The thermometer was read by means of a telescope.

The cathetometer used in the investigation was accurate to 0.02 mm. over the range required. It was fitted with a microscope objective (16 mm.), which enabled the meniscus in the fine capillary to be seen with ease, even at temperatures very close to the critical.

Preliminary work showed the necessity of having a very slight temperature gradient from the top of the bath to the bottom. This was attained by adjusting the positions of the heating elements, and regulating the rate of stirring. With such a temperature gradient, never permitted to exceed 0.1°C., the distillation of the liquid in an upward direction in the fine capillary was prevented.

Furthermore, it soon became evident that, having set the thermoregulator for a given temperature, several hours elapsed before equilibrium was established. Owing to the slow attainment of equilibrium, the bomb was usually permitted to remain overnight at the desired temperature, regulated within 0.2°C. Successive readings of the capillary rise were then taken every half hour, the temperature being held constant, by manual control, to within 0.03°C. Equilibrium was considered to be established when three successive readings, at half hour intervals, did not differ by an amount exceeding 0.04 mm.

Having made the required measurements of the capillary rise for methyl ether and propylene, the capillary tube was removed and calibrated over its entire length. This was done by introducing a short thread of mercury, and measuring its length at successive positions in the tube by means of a horizontal cathetometer. This mode of calibration has been shown by Richards and Carver (14) to be sufficiently accurate, the presence of a film of air between the mercury and glass not introducing an appreciable error. The length of the mercury thread at successive positions was ascertained with an error not exceeding 0.1%.

Since a balance sufficiently sensitive to weigh the quantity of mercury constituting the thread was not available, and since relative values of the surface tension were adequate, the diameter of the capillary was measured at one point with a microscope fitted with a micrometer eyepiece. The error did not exceed 1%, and as will be evident later, was considerably less than this amount. No difference in diameter was detectable by making measurements at right angles, indicating the absence of appreciable ellipticity in the bore.

From a knowledge of the relative values of the radius at various points in the capillary, as provided by the mercury thread calibration, the radius itself, corresponding to any position in the tube could be calculated from the value determined microscopically. From a calibration curve, the appropriate value for any experimentally determined capillary rise could be readily ascertained.

The densities of the liquid and vapor phases for methyl ether, necessary for the calculation of the surface tension, were taken from the data of Cardoso and Cappola (1). Data for the densities of liquid and gaseous propylene, over the temperature range employed in the present investigation, are not available in the literature. Consequently, these were determined; the method adopted and the results obtained will be discussed in a future paper dealing with the densities in the liquid and gaseous phases in the critical region.

Results and Discussion

The results obtained by the capillary rise method will be tabulated and discussed before those obtained by the ring method, since the former served as a basis for the calibration of the ring used in the latter method.

Capillary Rise Method

The surface tension was calculated for each liquid, at a given temperature, from the well-known formula relating the surface tension to the rise of a liquid in a capillary tube, where the angle of contact is zero, namely, $T=\frac{rgh(D-d)}{2}$, where T is the surface tension of the liquid in dyne/cm.; r is the radius of the capillary in cm.; g is the gravitational constant; h is the rise in cm., of the liquid in the capillary tube; and $D-d$ is the difference in density of liquid and vapor, at the temperature employed. The degree of accuracy sought did not necessitate a correction for the volume effect of the meniscus.

The results obtained for the surface tension of methyl ether are shown in Table I, and graphically represented in Fig. 2, while those for the surface tension of propylene are set forth in Table II, and graphically represented in Fig. 3. The experimental data for propylene are considered to be somewhat more accurate than those for methyl ether, owing to the greater facility with which the temperature could be controlled in the case of the former.

TABLE I

THE RELATION BETWEEN THE SURFACE TENSION OF METHYL ETHER AND TEMPERATURE

Temp., °C.	102.50	109.50	115.00	122.00	124.00	125.00
S.T., dyne/cm.	1.86	1.25	0.78	0.24	0.11	0.05

Although relative values of the surface tension were adequate for the solution of the problem originally conceived, the data obtained probably deviate less than 5.0% from the true values. This deviation, should it exist, is to be accounted for by the error arising in the microscopic ascertainment of the radius of the capillary at the one point, to which the radii at other points are referred. This method however has been adopted by Sugden (20) in much of his work on the surface tension of liquids. In any case, the relative values are considerably more accurate than the limit quoted above, and hence the conclusions drawn should be quite valid.

The variation of the surface tension with temperature at the critical temperature was the main objective. Whether or not this objective has been attained must be discussed from the actual observations which have been made. The question, of which the answer was sought, may be stated thus: is the tangent to the surface tension-temperature curve definitely at an angle of 90 degrees to the surface tension axis, at the critical temperature? The degree of certainty with which this question may be answered depends on the proximity to the critical temperature of the last point observed on the temperature scale, and the magnitude of its ordinate, these to be correlated to the experimental error involved. The last observation which could be made with certainty was approximately 1.5°C. below the critical temperature, with an ordinate value that could be ascertained with an error not exceeding 5%. Taking this point with those points preceding, and extrapolating on a semi-linear basis, there is a gradient which is not zero at the critical temperature.

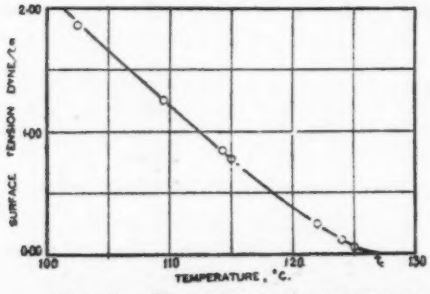

FIG. 2. *The surface tension-temperature relation for methyl ether.*

On the other hand, it is conceivable that determinations made above the last temperature attained might fit into an asymptotic surface tension-temperature curve at the critical tem-

TABLE II

THE RELATION BETWEEN THE SURFACE TENSION OF PROPYLENE AND TEMPERATURE

Temp., °C.	62.10	73.20	77.80	83.60	88.20	89.60	90.50
S.T., dyne/cm.	2.40	1.34	0.94	0.50	0.17	0.09	0.05

perature. Assuming this to be the case, a very marked inflexion in the surface tension-temperature curve would have to be admitted, this inflexion occurring quite close to the critical point. This is not inconceivable, since there are marked variations in density, with temperature, in this region; nevertheless, the results obtained would indicate the possible existence of pseudo-negative surface tension above the critical temperature. A negative surface tension is quite as impossible as a negative temperature on the absolute scale; the word "pseudo-negative" is used, therefore, to indicate a measurement which would be dependent upon a *visible* position of the meniscus above the critical point.

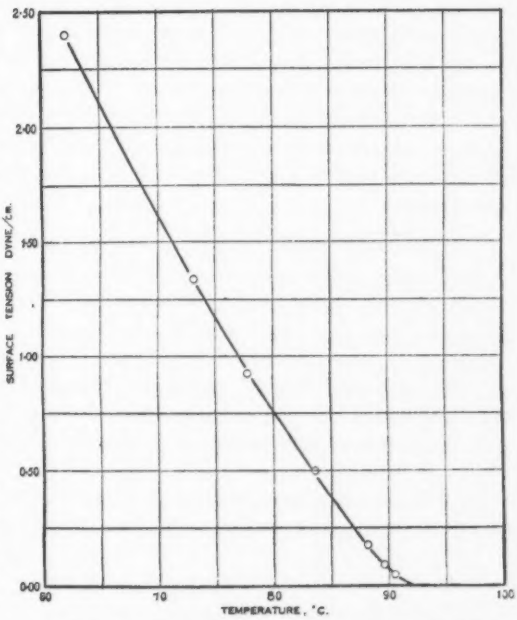

FIG. 3. *The surface tension–temperature relation for propylene.*

The meniscus is usually defined as a visible line of demarcation between the liquid and vapor phases; this implies a thickness such that its presence may be ascertained through the agency of visible light. This definition may well be extended, however, to include dimensions not detectable by the wave-lengths of ordinary, or visible, light. In so doing, the existence of surface energy is not denied; if a difference in density between two media persisted, the surface energy connected with the passage from that of the greater to that of the lesser density would be reflected in a definite angle of interception of the surface tension-temperature curve with the zero surface tension axis, even if the meniscus disappears in so far as optical detection is concerned.

From a consideration of the data obtained, the authors are of the opinion that a definite difference between the density of the liquid and that of the

vapor persists above the so-called critical temperature. This conclusion has been confirmed in an investigation of the densities in this region, the results of which will be communicated in a future paper.

Having applied the data to the solution of the original problem, as above, additional information of considerable value can be obtained from a few fundamental considerations. As pointed out previously, the available data for the surface tension of liquids near the critical point are very limited. Since the relative values of the surface tension obtained in this investigation are quite accurate, they can be profitably applied to the examination of various relations involving surface tension. The actual values of the constants, etc., which can be evaluated, are possibly slightly in error, although, as will become evident later, the calculation of these constants points to a high degree of accuracy, even in so far as the actual values of the surface tension are concerned.

(a) Molecular Surface Energies of Methyl Ether and Propylene

The molecular surface energies for methyl ether and propylene were calculated at each temperature, together with the Ramsay and Shields constant over the corresponding temperature intervals. The results obtained are shown in Table III. It is evident, from the rapid diminution in the value of the Ramsay and Shields constant for methyl ether, that their equation is not applicable at temperatures approaching the critical. At first glance, the value of k would appear to be considerably too low. Maass and Boomer (9) however report a decrease in the value of k for methyl ether from 2.12 at −42.0°C. to 1.76 at −10.6°C., which brings the values obtained in the present investigation in line with those at the lower temperatures.

TABLE III

THE MOLECULAR SURFACE ENERGIES AND THE RAMSAY AND SHIELDS AND KATAYAMA CONSTANTS FOR METHYL ETHER AND PROPYLENE

Temp., °C.	$S(M/D)^{\frac{2}{3}}$	k (R & S)	$S(M/D\text{-}d)^{\frac{2}{3}}$	k_1 Katayama	Temp., °C.	$S(M/D)^{\frac{2}{3}}$	k (R & S)	$S(M/D\text{-}d)^{\frac{2}{3}}$	k_1 Katayama
Methyl ether					*Propylene*				
102.50	38.64	1.67	44.15	1.72	62.10	52.0	1.97	58.8	2.09
109.50	26.93	1.73	32.07	1.87	73.20	30.2	1.80	35.6	1.93
115.00	17.40	1.66	21.76	1.92	77.80	21.9	1.70	26.7	1.84
122.00	5.77	1.57	8.28	2.08	83.60	12.1	1.65	16.0	1.95
124.00	2.63	1.36	4.11	1.88	88.20	4.5	1.43	7.0	1.89
125.00	1.28		2.23		89.60	2.5	1.32	4.3	1.92
					90.50	1.3		2.6	

In the case of propylene also the rapid diminution in the value of k near the critical temperature is evident, and is quite in line with the results obtained by Maass and Wright (10) at lower temperatures.

The validity of the equation developed by Katayama (6) has been subjected to a critical examination by means of the results obtained in this investigation.

His equation is of the form, $S(M/D-d)^{\frac{1}{3}} = k_1(T_c-T)$, where S is the surface tension of the liquid; M is the molecular weight of the substance; $D-d$ is the difference in densities of liquid and vapor at temperature T; and k_1 is Katayama's constant.

The values for the molecular surface energies calculated from this equation, also the values of k_1, are shown in Table III for methyl ether and propylene. It is of interest to note that the value of k_1 remains quite constant, even at temperatures very close to the critical, indicating the advantages of the Katayama equation over the classical equation of Ramsay and Shields.

(*b*) *The Relation Between Temperature and the Surface Tensions of Methyl Ether and Propylene*

In 1894, van der Waals suggested the following formula to express the variation of surface tension with temperature:

$$S = K_1 T_c V_c^{-\frac{2}{3}} (1-T_r)^b = K_2 T_c^{-\frac{1}{3}} P_c (1-T_r)^b.$$

Here T_c, V_c, and P_c are the critical temperature, volume, and pressure respectively, and T_r the reduced temperature. K_1 and K_2 and b are universal constants. Sugden (20) has obtained this equation in the form, $S = S_o(1-T_r)^{\frac{3}{2}}$, where S_o represents the surface tension of the supercooled liquid at the absolute zero. If the equation accurately represents the variation of the surface tension with temperature, a plot of $S^{\frac{1}{4}}$ against temperature should give a straight line. Sugden has found that the equation holds accurately, and a linear relation is obtained, provided the liquid is not associated. With associated liquids, the graph takes on a decided curvature.

In Fig. 4 are plotted values of $S^{\frac{1}{4}}$ for methyl ether and propylene, for values of S ascertained from Figs. 1 and 2, at 5°C. intervals of temperature. It is quite evident that the equation is valid for the two liquids under investigation, from which it is to be concluded that methyl ether and propylene are not associated at the temperatures employed.

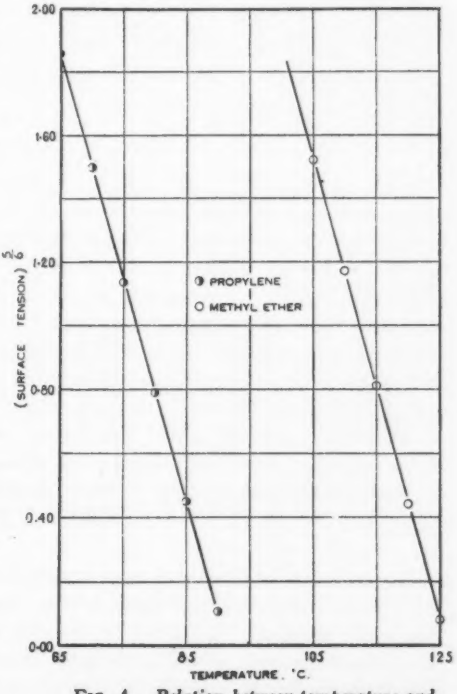

FIG. 4. *Relation between temperature and* $(S.T.)^{\frac{1}{4}}$.

(c) The Relation Between the Surface Tensions and Densities of Methyl Ether and Propylene

A relation between surface tension and density has been obtained by Macleod (11). His equation is of the form $S = C(D-d)^4$, where D and d are the densities of the liquid and vapor, and S is the surface tension. The constant, C, has been found invariable with temperature up to the neighborhood of the critical temperature.

The values of $C^{\frac{1}{4}}$ at various temperatures have been calculated for methyl ether and propylene, and are shown in Table IV. For methyl ether, there seems to be no definite and progressive increase in the value of $C^{\frac{1}{4}}$, but a marked and progressive increase is noticeable in the case of propylene. An increase in the value of this constant has been noticed near the critical point in the cases of benzene and chlorobenzene, but not in the case of carbon tetrachloride. The tendency for the value of $C^{\frac{1}{4}}$ to increase near the critical temperature has been attributed to errors in the surface tension and density measurements in this region. An alternative explanation will be postulated in connection with the parachors of these substances.

(d) The Parachors of Methyl Ether and Propylene

The parachors of methyl ether and propylene at various temperatures are shown in Table IV. Although the parachor of methyl ether does not deviate largely or consistently from the theoretical value of 132.2, that of propylene shows a marked increase over the theoretical value of 140.2, as the critical temperature is approached.

TABLE IV

THE MACLEOD CONSTANT AND THE PARACHOR FOR METHYL ETHER AND PROPYLENE

Temp., °C.	$C^{\frac{1}{4}}$	$MC^{\frac{1}{4}}$*	Temp., °C.	$C^{\frac{1}{4}}$	$MC^{\frac{1}{4}}$**
Methyl ether			*Propylene*		
			62.10	3.60	151.2†
102.50	2.94	136.0	73.20	3.51	147.4
109.50	2.99	137.4	77.80	3.53	148.3
115.00	3.02	138.9	83.60	3.64	152.9
122.00	3.08	141.8	88.20	3.93	165.0
124.00	3.03	139.3	89.60	4.29	180.2
125.00	2.96	136.2	90.50	4.68	196.5

*$MC^{\frac{1}{4}}$ (theoretical) = 132.2. **$MC^{\frac{1}{4}}$ (theoretical) = 140.2. †*This value is probably too high, owing to the necessity of extrapolating the density data for the calculation of the surface tension.*

To account for the large and progressive deviation of the propylene parachor from the theoretical value on the basis of experimental error alone does not seem justified. Like the increase in the Macleod constants for benzene and chlorobenzene, which have been accounted for on this basis by Sugden, the increase in the parachors of these substances is essentially explained in the same way, if no alternative explanation can be offered. It is significant,

however, that for benzene and chlorobenzene, as for propylene, the progressive increase in parachor becomes noticeable at temperatures as remote as 50-70° from the critical point. It is highly improbable that the errors in density and surface tension measurements in this temperature range would be sufficiently large and systematic to account for the very definite and progressive increase in the parachors of propylene, benzene, and chlorobenzene, whereas in the cases of methyl ether and carbon tetrachloride no such increase is evident.

Owing to the unfortunate lack of sufficient data from which to draw definite conclusions, it is not possible to advance a general explanation with a large degree of certainty. From the few data available, however, it would seem that those molecules which possess a multiple bond in their structure, as a consequence of which considerable electronic interaction might be expected, show an increase in parachor with temperature. On the other hand, those molecules which possess no multiple linkages in their constitution do not exhibit a tendency towards an increase in parachor with a rise of temperature. In this latter type of molecule, little or no electronic interaction would be expected.

The increase in parachor due to the presence of double or triple bonds, or to ring systems, has been accounted for on the basis of electronic interaction. It postulated that, as a result of this interaction where the electrons are probably congested, as in the case of a double bond, the tendency is towards an expansion of the molecule. This is to be regarded as analogous to an increase in parachor, since evidence points to a direct proportionality between the parachor of a molecule and its range of molecular action, which is of the same order of magnitude as the molecular diameter.

By a similar argument, it is conceivable that an increase in temperature would result in an increased vibrational energy of the molecule, in an increased electronic interaction, and thus in an increased sphere of molecular influence. This would be evident in those molecules in which electronic interaction is pronounced, whereas, in the case of molecules containing only single bonds, and consequently negligible interaction between the electrons, the coefficient of parachor increase with temperature would also be negligible. In the case of the former type of molecule, the increased molecular action would be reflected in an increased parachor. This, in turn, might be interpreted as an increase in unsaturation, since expansion of the molecule, due to augmented electronic interaction, would increase the tendency of the unsaturated carbon atoms to share electrons.

B. Ring Method

In determining the utility of this method for the determination of surface tension near the critical temperature, the surface tension-temperature relations for methyl ether have been investigated over a range of temperature from 111.5°C. to 119.2°C. A curve of the extensions of the spiral observed at five different temperatures was first plotted. Reference was then made to

the curve obtained for the surface tension of methyl ether by the capillary rise method, and the surface tension at 111.5°C. ascertained. Using the well-known formula for the ring method, $T = \frac{mg}{2L}$, where T is the surface tension of the liquid, m is the force in grams necessary to remove the ring from the surface, g is the gravity constant, and L is the circumference of the ring, the value of L was calculated by substitution of the value of T at 111.5°C. Then, since m corresponding to any other temperature could be ascertained from the observed extension of the calibrated quartz spiral, the value of the surface tension at any other temperature in the range studied was readily calculable.

In Table V is shown the agreement between the values obtained with the ring method and those read from the curve obtained by the capillary rise method, for two-degree intervals between 110.0°C. and 120.0°C., a small extrapolation being necessary to obtain these two particular values. The values show remarkably good agreement, even at the higher temperatures.

TABLE V

COMPARISON OF THE SURFACE TENSIONS OF METHYL ETHER BY THE RING AND CAPILLARY RISE METHODS

Temp., °C.	110.00	112.00	114.00	116.00	118.00	120.00
S.T. (ring), dyne/cm.	1.16	1.04	0.89	0.71	0.54	0.39
S.T. (cap. rise), dyne/cm.	1.21	1.04	0.88	0.70	0.54	0.38

Although the data are not comprehensive, they serve to indicate two points of significance. The ring method is independent of the angle of contact which the liquid makes with the glass. The results obtained by the capillary rise method were calculated on the assumption that the angle of contact was zero. Since these results agree with those obtained by the ring method, it must be concluded that the angle of contact between methyl ether and glass is zero. Furthermore, although the ring method was abandoned in favor of the capillary rise method, purely from the standpoint of manipulative ease, the results obtained by the former method do indicate the feasibility of applying this method to the determination of surface tension near the critical point. With some modifications, it is conceivable that the method would be much more readily applicable than in its present form. That the ring method possesses certain advantages for work in the critical region is undoubtedly true. Should it prove to be the case that a meniscus persists above the critical point, although optically indetectable, the ring method, or a modification thereof, would seem to be the most suitable method for its detection, and for the determination of the surface energy involved. The fact that the method is independent of the angle of contact is also an advantage, as is also the fact that the densities of the liquid and vapor phases need not be known.

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THE YELLOW COLORING MATTER OF KHAPLI WHEAT, *TRITICUM DICOCCUM*

III. THE CONSTITUTION OF TRICIN[1]

By J. Ansel Anderson[2]

Abstract

Tricin is identical with 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone which has been synthesized by the action of concentrated sulphuric acid on 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone. The constitution has been confirmed by fusion with alkali which yielded syringic acid and phloroglucinol.

The isolation of tricin, a trihydroxydimethoxyflavone, from Khapli wheat leaves was reported by Anderson and Perkin (2). In a later communication (1) it was shown that tricetin, the compound formed by demethylation of tricin, was 5,7,3′,4′,5′-pentahydroxyflavone. In view of the weak dyeing properties of tricin as compared with those of tricetin, it at once appeared altogether likely that in the former, two of the three hydroxyl groups in the 3′-, 4′- and 5′-positions were methylated. In these circumstances it seemed most probable that tricin contained the syringic nucleus, which occurs quite commonly in compounds of natural origin. The possibility that the methoxyl groups occurred in the 3′- and 4′-positions was, however, by no means negligible since this condition occurs in the isoflavone, irigenin. Synthetic experiments designed to determine the constitution of tricin were not undertaken immediately since the isolation of a further supply of tricin was expected during the examination of a fresh supply of wheat leaves, thus making it possible to proceed by the more logical method of determining the hydrolyzation products of tricin before attempting its synthesis. In the meantime, Venkataraman and Gulati have formed the opinion that tricin contains the syringic nucleus and have published a note (5) declaring their intention of synthesizing 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone by the interaction of phloracetophenone and O-benzylsyringic anhydride.

Approximately one gram of fairly pure tricin was isolated recently from Khapli wheat leaves. Attempts to hydrolyze the compound with potassium hydroxide in 80% alcohol failed owing to the relative insolubility of the potassium salt in this medium. The hydrolysis of the small quantity of tricin remaining after the completion of the above experiments, was effected by the use of 50% alcohol, in which the compound remained dissolved. The results were by no means clearcut but offered some indications that tricin contained the syringic nucleus and was therefore tricetin 3′,5′-dimethyl ether (II).

[1] *Manuscript received July 4, 1933.*
Contribution from the National Research Laboratories, Ottawa, Canada.
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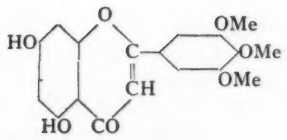
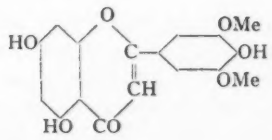


(I)



(II)

Synthetic experiments were undertaken pending the isolation of more tricin. A quantity of 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone (I) being available, an attempt was made to demethylate the group in the 4′-position by means of sulphuric acid under conditions similar to those used for the preparation of syringic acid from 3,4,5-trimethoxybenzoic acid (3, p. 1553). The first attempt proved successful and slight variation of the conditions in later experiments led to a fair yield of tricetin 3′,5′-dimethyl ether (II). Heap and Robinson (4) attempted the preparation of syringetin by this method but were unable to find the conditions for its successful use.

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone (II) proved to be identical with tricin in all respects. Since tricin had not proved amenable to alkaline hydrolysis, confirmation of the structure was attempted by fusion with alkali. The methoxyl groups were quite stable under the conditions employed and syringic acid and phloroglucinol were isolated.

The water-soluble coloring matters of wheat leaves are still under investigation and the examination of other organic constituents of the plant has also been undertaken.

Experimental

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone (Tricin)

5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone (1) (3 gm.) was dissolved in sulphuric acid (4.5 cc. of 96–98%) and heated at 60° C. A small quantity of orange precipitate separated gradually. After 6 hr. the mixture was transferred to water (200 cc.). The orange-yellow, lumpy precipitate which separated was broken up and the mixture was heated on the steam bath for 0.75 hr. The solid was collected, washed with water and crystallized from hot dilute acetic acid (1.43 gm.), m.p. 288-289° C.* Three recrystallizations from acetic acid yielded clusters of pale yellow, glistening needles, m.p. 291-292° C. Analysis: Calcd. for $C_{17}H_{14}O_7$; C, 61.8; H, 4.3; CH_3O, 18.8%. Found after drying: C, 61.6; H, 4.3; CH_3O, 18.3%.

Previous preparations of natural tricin had softened and melted indefinitely over a comparatively wide range. Some trouble was experienced in preparing a sample melting sharply at as high a temperature as the synthetic specimen. This was finally effected by careful deacetylation of the pure acetyl derivative and recrystallization from acetic acid. A mixture of equal quantities of the synthetic and natural compounds melted at 291-292° C.

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone is fairly readily soluble in ethyl alcohol, methyl alcohol and acetic acid; soluble in ethyl acetate; rather

*All melting points are corrected.

sparingly soluble in ether; very sparingly soluble in chloroform and toluene; almost insoluble in benzene and water; and insoluble in ligroin. It dissolves in concentrated sulphuric acid and in dilute aqueous sodium hydroxide to give bright yellow solutions. In alcoholic solution it gives a reddish-brown color with a trace of alcoholic ferric chloride and a dark olive-brown with more concentrated reagent. With alcoholic lead acetate it gives a bright yellow precipitate and with alcoholic potassium acetate a pale yellow precipitate. No color changes could be obtained by treating an alcoholic solution of the flavone with very dilute alcoholic potassium hydroxide. A hot concentrated solution of the flavone in glacial acetic acid, when treated with a trace of concentrated sulphuric acid and cooled, yielded clusters of minute, bright orange needles of the oxonium salt. The coloring matter is a very weak dye. It gives a pale lemon-yellow on aluminium-, a pale brown on iron-, and a greenish-yellow on chromium-mordanted wool. These properties are identical with those of tricin.

5,7,4′-Triacetoxy-3′,5′-dimethoxyflavone

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone (0.15 gm.) was refluxed for 1 hr. with acetic anhydride (0.6 cc.) containing a trace of pyridine. The acetyl compound crystallized out on cooling (0.16 gm.). It was recrystallized from acetic anhydride and again from a mixture of alcohol and acetic acid and was thus obtained in colorless needles, m.p. 251-254° C. Analysis: Calcd. for $C_{23}H_{20}O_{10}$: C, 60.5; H, 4.4; CH_3O, 13.6%. Found: C, 60.6; H, 4.5; CH_3O, 13.7%. The melting point was unchanged when the compound was mixed with an equal quantity of triacetyl tricin.

Alkaline Fusion

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone (1.5 gm.) was heated for 5 min. at 200–210° C. with potassium hydroxide (15 gm.) and a few drops of water. The melt was dissolved in water and acidified with hydrochloric acid. After filtering from a trace of dark precipitate the solution was thoroughly extracted with ether. The residue which remained after evaporation of the ether was taken up in a solution of sodium bicarbonate, filtered from a trace of yellow insoluble matter, and again extracted with ether (A).

The extracted aqueous solution was acidified with dilute hydrochloric acid. A crystalline precipitate (B) of almost colorless needles separated immediately. This was collected and the filtrate was again extracted with ether (C).

Isolation and identification of phloroglucinol. The ether extract (A) was dried and evaporated to dryness. The residue was taken up in a little water, filtered from a minute trace of insoluble matter and the filtrate evaporated to dryness *in vacuo*. A crystalline residue remained (0.12 gm.), m.p. 216–218° C. A small quantity of this compound when dissolved in water gave a bluish-violet color with aqueous ferric chloride solution.

The compound (0.1 gm.) was dissolved in a solution of potassium hydroxide (0.155 gm.) in water (0.5 cc.). The solution was cooled in melting ice and a

small piece of ice was added. Acetic anhydride (0.24 cc.) was then added with vigorous shaking. An almost colorless precipitate separated immediately. It was collected, washed with water and dried (0.11 gm.). Recrystallization from alcohol yielded minute, colorless needles (0.07 gm.) which melted at 105-106° C. and at the same temperature when mixed with an equal quantity of triacetyl phloroglucinol.

Isolation and identification of syringic acid. The precipitate (B) was washed with a little water and dried (0.43 gm.), m.p. 208-209° C. It was recrystallized from water (Norit) and was thus obtained in colorless, silky needles which melted at 210-211° C. and at the same temperature when mixed with an equal quantity of syringic acid.

The ether extract (C) was dried and evaporated to dryness. The residue (0.18 gm.) by recrystallization from water yielded a small quantity of syringic acid. Color reactions indicated the presence of a trace of gallic acid.

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ON THE SPECIFIC HEAT OF COPPER FROM −78° TO 0° C.[1]

BY S. M. DOCKERTY[2]

Abstract

This paper is a continuation of recent work by H. L. Bronson, H. M. Chisholm, and the author (3) on the specific heats of tungsten, molybdenum, and copper from 0° to 500° C. The "method of electrical heating" and adiabatic calorimetry have been extended to determine the specific heat of copper from −78° to 0° C.

The equation previously given for the specific heat of copper contained only the first two terms of the Debye expansion and was found not to hold below −30° C. The following equation containing four terms of the Debye expansion fits the experimental curve from −78° to 500° C. with a maximum deviation of only about 0.05%,

$$C_p = .3889 + 5.65 \times 10^{-4}T - \frac{2000}{T^2}\left(1 - \frac{321^2}{287^2} + \frac{321^4}{9107^4}\right),$$

where the units are joules per gram per °K. The constants of this equation were determined empirically and their close relation to theoretically expected values has been discussed.

Introduction

In the previous work an adiabatic calorimeter of the Richards' type was used except that the usual water calorimeter was replaced by solid copper calorimeters from 1 to 5 kgm. in mass. The specific heat of copper from −5° to 110° C. was determined directly by heating these calorimeters electrically under adiabatic conditions. The current and voltage were measured with a potentiometer, and the rise in temperature with a platinum thermometer and bridge of the Callendar-Barnes type (1). The "copper equivalent" of each calorimeter was accurately known since the non-copper components were only about 0.5%. All measurements were thus made directly and with high precision; the method is therefore practically free from the errors and difficulties experienced in the use of the "method of mixtures." It also has the great advantage of giving the average specific heat over a small range of temperature and is particularly well suited for the measurement of specific heat at, or near, room temperature where the "method of mixtures" is quite unsuitable. The slope of the specific heat-temperature curve thus obtained increased more and more rapidly as the temperature decreased to 0° C. This is of particular interest from a theoretical standpoint and it seemed desirable to extend this accurate method of measurement to still lower temperatures.

In the present investigation it has been found possible to overcome the difficulties of adiabatic calorimetry at temperatures down to −78° C., and the precision of the results obtained compare favorably with those obtained above room temperature in the previous investigation (3).

[1] *Manuscript received May 25, 1933.*
Contribution from the Department of Physics, Dalhousie University, Halifax, Nova Scotia, Canada.

[2] *Postgraduate student, Dalhousie University, and holder of a bursary under the National Research Council of Canada.*

Apparatus and Experimental Method

Adiabatic Calorimeter

It was not thought practicable with the apparatus available to use a liquid bath for the low temperature work, so a thick cast-copper shell was made to replace the oil bath and jacket of the adiabatic calorimeter used in the previous experiments. This shell (*A*, Fig. 1) was made of such dimensions that it was suitable for the accommodation of the 1100-gm. copper calorimeter used in the previous investigation. The body and bottom are in one piece, while between the cover and body there is a carefully ground joint. Three separate heating coils were wound in grooves on the sides and on the ends and the whole was thermally insulated from the outside by cotton wool packing between the shell and the container (*D*, Fig. 1).

It was hoped that the high conductivity of copper and the thickness of the shell would ensure proper adiabatic conditions over the inner surface of the shell even when there was considerable variation in temperature over the outer surface. This however was not found to be the case and variations were present of a size which indicated that the shell, which was not of pure copper, had a conductivity considerably less than was expected. It was therefore necessary to adjust the heating and thermal insulation on the outside of the shell with considerable care.

Differences in temperature between points on the inside of the shell were measured by means of a thermocouple, the junctions of which could be moved from point to point over the inner surface.

Temperature inequalities on the inner surface between sides, bottom, and top, caused by faulty distribution of heat losses, were largely eliminated by adjusting the amount of thermal insulation on the sides and ends. This was accomplished for the ends by adjusting the spaces between the top and bot-

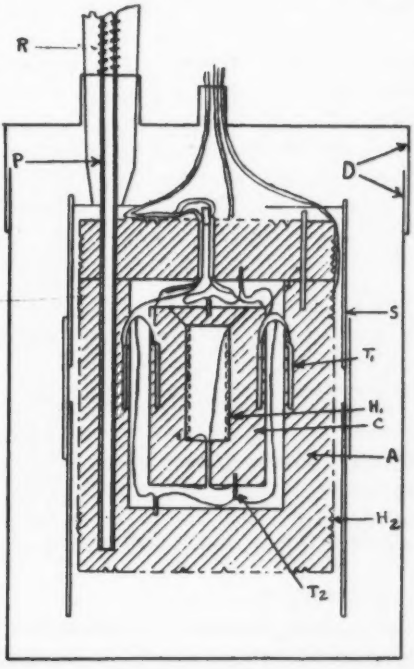

FIG. 1. *Adiabatic calorimeter assembled.*

FIG. 2. *Platinum thermometer.*

tom of the shell and the container, and for the sides by varying the length of the brass shield (*S*, Fig. 1) and thus changing the effective area of the sides. These adjustments were successfully made when there was a temperature difference of 90° C. between the shell and the air of the room. The heat loss to the outside for such a temperature difference was the equivalent of about 20 watts. The fraction of the current in each heating coil was then so adjusted that uniformity in temperature over the inner surface was also obtained when the shell was rising in temperature at the rate of 0.25° C. per minute (the usual rate during an experiment).

The Copper Specimen

The specimen of copper used in this work was the 1100-gm. cold-rolled copper calorimeter used in the previous experiments, and has the form and dimensions shown in *C*, Fig. 1. As the calorimeter was 99.5% copper there was no difficulty in calculating the "copper equivalent" to the required degree of accuracy. The weights of the various parts and their calculated "copper equivalents" are given in Table I.

TABLE I

WEIGHTS OF CALORIMETER PARTS AND THEIR CALCULATED COPPER EQUIVALENTS

Part of calorimeter	Weight, gm.	Copper equiv., gm.	Part of calorimeter	Weight, gm.	Copper equiv., gm.
Copper calorimeter	1128.95	1128.95	Aluminium foil	0.04	0.10
Brass hinges and screws	4.10	4.04	Copper cylinder for heating coil	15.37	15.37
Steel supporting wires	0.03	0.04	Manganin wire for heating coil	2.54	2.63
Brass tubing for thermocouples	2.43	2.40	Cotton insulation for heating coil	0.965	3.92
Wire for thermocouples	0.16	0.16	Copper leads for heating coil	0.46	0.46
Cotton insulation on thermocouples	0.15	0.60	Air correction		0.40
Cement for thermocouples	0.15	0.30	Total		1159.4

Although there is some uncertainty in the "copper equivalent" of the cotton insulation, an error of as much as 0.5 gm. is highly improbable.

The thermocouple, current, and potential leads were brought out through a hole in the top of the shell and were run for some distance along its top to prevent heat losses from the inner calorimeter along these wires.

Differential Thermocouples

Temperature equality between the calorimeter and the shell was assured by an eight-junction thermocouple of No. 34 copper and No. 32 constantin wire. The junctions of this thermocouple were set in plastic cement in brass tubes and were arranged in two pairs of three-junction and two pairs of one-

junction couples. These were mounted on the sides and ends of the calorimeter and shell in the ratio 6 : 1 : 1. Since this was about the ratio of the areas involved the arrangement should have largely eliminated the effects of any temperature differences on the inner surface of the shell. Opposite junctions were similarly mounted (T_1 and T_2, Fig. 1) in order to eliminate the effects of lag and of possible discrepancies arising from the shortness of some of the junctions. With a sensitive galvanometer this thermocouple gave a deflection of 8 mm. for a temperature difference of 0.001° C. between the calorimeter and shell.

Measurement of Temperature

The temperature of the copper calorimeter was found by measuring the temperature of the shell when the differential thermocouples indicated temperature equality between the two. This was done by means of a platinum thermometer of the form shown in Fig. 2. This thermometer had a resistance of 20 ohms at 0° C. and the sensitivity of the bridge was such that settings could be made with confidence to within 0.001° C. The upper part of the thermometer was of Pyrex glass and was joined by a piece of heavy rubber tubing to the nickel stem. This nickel tube was 8 mm. in diameter and fitted neatly into a hole 14 cm. deep in the copper shell (P, Fig. 1). The exposed stem was carefully wrapped with cotton wool to prevent heat losses from the shell along the metal stem. In spite of this, however, it was found that when the temperature of the shell was considerably above that of the room enough heat was conducted up the stem to upset the thermal adjustments previously made. This difficulty was overcome by winding a heating coil (R, Fig. 1) on the upper part of the nickel stem and adjusting the heating current till the thermal adjustments were the same as they were before the thermometer was inserted. A curve was drawn giving the required value of this current for any particular temperature difference between the shell and the outside surroundings.

Constants of the Platinum Thermometer

In using the platinum thermometer for the measurement of temperature the following equations are internationally accepted (4):

$$R_t = R_0(1 + At + Bt^2) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 0° \text{ to } 660° \text{ C.}$$

$$R_t = R_0(1 + At + Bt^2 + C(t - 100)t^3) \quad \ldots\ldots\ldots \quad 0° \text{ to } -190° \text{ C.}$$

where t is in ° C. If $\dfrac{R_t - R_0}{R_{100} - R_0} \times 100$ is denoted by t_p we obtain equations in the usual convenient form for temperature calculation:

$$t - t_p = \delta\left(\frac{t^2}{100^2} - \frac{t}{100}\right) \quad \ldots\ldots\ldots\ldots\ldots \quad 0° \text{ to } 660° \text{ C.}$$

$$t - t_p = \delta\left(\frac{t^2}{100^2} - \frac{t}{100}\right) + \gamma(t - 100)t^3 \quad \ldots\ldots\ldots \quad 0° \text{ to } -190° \text{ C.}$$

The constants δ and γ, determined from the boiling points of water, sulphur, and liquid oxygen, were found to be:—$\delta = 1.52$—$\gamma = 9.3 \times 10^{-10}$.

Preliminary Experiments Above Room Temperature

Before proceeding to the low temperature measurements it was considered desirable to test the apparatus over a range which had been previously investigated. Specific heat determinations were therefore made at 10° intervals from 20° to 110° C. with the apparatus in open air. The experimental procedure for such measurements has been fully discussed in the previous paper (3). No difficulty was found in maintaining temperature equality between the calorimeter and jacket to within 0.003° C. while the calorimeter was being heated and calculation showed that a difference of 0.01° during the whole time of an experiment would introduce an error of less than 0.1%. At the end of an experiment not less than 15 min. was allowed for the copper shell to reach equilibrium before the platinum thermometer was read. During these determinations the platinum thermometer stem was heated in the manner previously discussed. The results obtained are given in Column 2, Table II. In Column 3 are shown the results previously obtained when an oil bath was used. In Column 5 are values of I^2, the mean of the squares of the heating currents necessary to maintain the shell at a constant temperature at the beginning and end of an experiment.

TABLE II

SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Mean temp., °C.	Specific heat		Diff.	I^2	Correction	Corr. value
	Experimental	Former results				
25.50	0.3842	0.38415	0.00005	0.007	0.00005	0.38415
36.58	0.3863	0.3863	0.00000	0.020	0.0001	0.3862
47.58	0.38835	0.38825	0.00010	0.035	0.00015	0.3882
58.52	0.39035	0.39005	0.00030	0.050	0.0002	0.39015
69.40	0.3920	0.39175	0.00025	0.064	0.00025	0.39175
80.24	0.3937	0.3933	0.00040	0.078	0.0003	0.3934
91.02	0.3952	0.39485	0.00035	0.095	0.0004	0.3948
101.75	0.3967	0.39625	0.00045	0.110	0.00045	0.39625
*105.17	0.39715	0.3967	0.00045	0.098	0.0004	0.39675
106.20	0.3974	0.39685	0.00055	0.118	0.0005	0.3969
*115.91	0.39845	0.3980	0.00045	0.110	0.00045	0.3980

**Taken after low temperature determinations.*

It is evident from the above figures that the deviation of the present experimental values from the former results is approximately proportional to I^2. The corrections in Column 6 are calculated as follows:—

Correction $= \dfrac{I^2 \times 0.00045}{0.110}$. This is of course done on the assumption that the original experimental curve was correct and that the differences in Column 4 are due to inequalities in temperature over the inner surface of the shell. Such inequalities would be proportional to the heat losses from the shell at any temperature and hence to I^2 which is a measure of these losses. Further

confirmation of the fact that the differences of Column 4 are real and that the observed values should be corrected as above will be shown later in connection with the low temperature measurements. Undoubtedly these corrections could be reduced by readjustment of the heating and thermal insulation, but it did not seem worth while when the maximum correction was only about 0.1%.

The Specific Heat of Copper between −78° and 30°C.

For the low temperature work the apparatus was placed in a thermally insulated receptacle and solid carbon dioxide was packed around the container *D*. The shell was then cooled rapidly to −120° C. by means of liquid air so that the temperature of the inner calorimeter was lowered to −78° C. in about one hour. When this point was reached the temperature of the calorimeter and shell were made equal and the platinum thermometer read. Specific heat determinations were then made, as before, at 10° intervals from −78° to 30° C. The time required for these measurements was only about 11 hr., which indicates the convenience of the method. The apparatus was about as easy to operate at this temperature as when working above room temperature. After the carbon dioxide had evaporated two determinations were made above 100° C. without disturbing the apparatus. These two points (marked with an asterisk in Table II) are in complete agreement with those obtained when the apparatus was in the open air. Since the surroundings of the container and of the exposed stem of the platinum thermometer were maintained at −78° C. during the low temperature experiments, the thermal conditions were similar to those obtained when working above room temperature with the container in the open air. The corrections in Table II should therefore be equally applicable to the lower range.

In Column 2 of Table III are given the values obtained for the specific heat of copper at various temperatures from −78° to 30° C., while in Column 3 are the corresponding values from the experimental curve of the previous

TABLE III

SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Mean temp., °C.	Specific heat		I^2	Correction	Corr. value
	Experimental	Former results			
−72.00	0.35495		0.012	0.00005	0.3549
−60.70	0.3599		0.027	0.0001	0.3598
−50.11	0.3641		0.035	0.00015	0.36395
−38.90	0.36775		0.044	0.00017	0.3676
−27.30	0.37145		0.055	0.00022	0.37125
−16.50	0.37465		0.065	0.00025	0.3744
−5.80	0.37745	0.37715	0.073	0.0003	0.37715
5.54	0.38005	0.3799	0.084	0.00033	0.3797
16.08	0.3826	0.38225	0.093	0.00037	0.38225
25.84	0.38465	0.38425	0.102	0.0004	0.38425

investigation (3). Columns 4 and 5 contain the values of I^2 and of the corrections calculated exactly as in Table II. The fact that the corrected values around room temperature agree, within the limits of experimental error, with those of Column 3 and also with the lowest value in Column 2, Table II, would seem to justify the method of calculating the corrections.

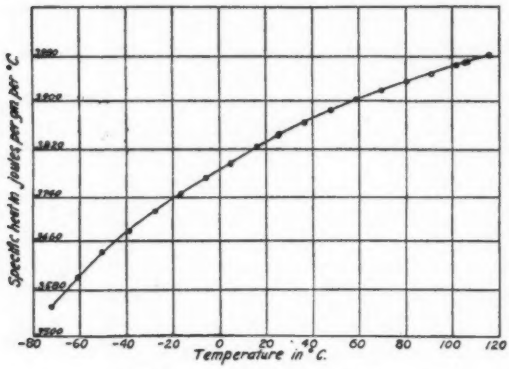

FIG. 3. *Specific heat of copper.*

When the values for the specific heat of copper given by the last columns of Tables II and III were plotted to a large scale only one point was found to lie off a smooth curve by as much as 1 part in 2000. This curve on a much smaller scale is shown in Fig. 3.

A comparison of the results obtained from −5° to 110° C. using the present type of adiabatic calorimeter with those obtained in the previous investigation indicates that the copper shell is a satisfactory substitute for the bath and jacket of the calorimeter previously used. There is nothing to indicate that the results obtained from −78° to 0° C. are less accurate or less reliable than those for the range 0° to 120° C. It would seem, therefore, that this type of apparatus might be successfully used for accurate specific heat determinations at still lower temperatures.

Equations for the Specific Heat of Copper

In the previous paper (3) it was pointed out that theoretical considerations suggested that an equation for the specific heat of metals should have the form:

$$C_p = A + BT - \frac{C}{T^2} \qquad (a)$$

where T is in degrees K. The first and last terms represent the first two terms of the Debye (5) expansion for C_v while the middle term represents $C_p - C_v$. The constants of this equation for the same copper calorimeter as used in the present experiments were determined empirically with the following results:

$$C_p = .3884 + 5.69 \times 10^{-5}T - \frac{1890}{T^2} \qquad (9J)^*$$

in which the units are joules per gram per degree K. This equation fitted the experimental points from −5° to 110° C. with an average deviation of less than 0.05%. The equations obtained for the specific heat of other specimens of copper, even up to 500° C., differed from Equation (9*J*) only

**This equation number used in the previous paper.*

in the first term and there by not more than 0.2%. We may therefore conclude that the *experimental* values of the specific heat of the copper calorimeter between 0° and 500° C. can be calculated from Equation (9*J*). Variations of a few tenths of 1% in the specific heat of different samples of copper are not surprising in the light of recent work by Jaeger, Rosenbohm, and Bottema (7), who have found that the specific heats of annealed and unannealed samples of both platinum and copper differed considerably, the former by as much as 2%.

In Column 2 of Table IV are shown the values of the specific heat of copper from −78° to 120° C. taken from the present experimental curve while Column 3 shows the values calculated by Equation 9*J* from −78° to 500° C. A comparison of Columns 2 and 3 shows that Equation 9*J* and the experimental curve are in very close agreement down to 250° *K*. but that they diverge rapidly below this point.

An examination of the magnitude of successive terms of Debye's (5) expansion:

$$C_v = C_\infty - \frac{C_\infty \theta^2}{20T^2} + \frac{C_\infty \theta^4}{560T^4} - \frac{C_\infty \theta^6}{18144T^6} + \ldots .$$

indicates that for temperatures of 200° *K*. the third and even the fourth term cannot be neglected and that Equation (*a*) should have the form:

$$C_p = C_\infty + BT - \frac{C_\infty \theta^2}{20T^2}\left(1 - \frac{\theta^2}{28T^2} + \frac{\theta^4}{910T^4}\right) \qquad (b)$$

The constants of this equation were determined empirically from the value of C_p at 200° *K*. given in Column 2, and the values at 400° and 800° *K*. given in Column 3 with the following results:

$$C_p = 0.3889 + 5.65 \times 10^{-5}T - \frac{2000}{T^2}\left(1 - \frac{321^2}{28T^2} + \frac{321^4}{910T^4}\right) \qquad (1)$$

The values calculated by this equation (Column 4, Table IV) agree closely with those in Column 3 above 300° *K*., while the maximum deviation from the experimental values of Column 2 is only about 0.05% at 250° *K*. Although Equation (1) fits the experimental curve remarkably well over a wide temperature range it is not entirely satisfactory from a theoretical standpoint. It was assumed that $C_p - C_v$ could be represented by a term BT, whereas thermodynamic theory indicates that it should have the form $\frac{9\alpha^2 V}{K} T$, where V is the specific volume and K the compressibility, both of which vary little with the temperature; α is the coefficient of linear expansion which Grüneisen (6) has shown to be directly proportional to C_v. Thus $C_p - C_v$ should have the form GC_v^2T. Since the ratio of C_p to C_v is approximately constant, and since C_p is the quantity determined experimentally, it is more convenient to express $C_p - C_v$ as $G'C_p^2T$ which is the form commonly given in standard text books. For copper the value of G' in such cases is given as 1.3×10^{-5} if the units are calories per mole, or 2.0×10^{-4} if the units are joules per gram. If C_v is used in the above formula instead of C_p, and the ratio of C_p to C_v taken as 1.025 the constant G becomes 2.1×10^{-4}.

Born and Brody (2) have shown that Debye's expression for C_v should contain a term DC_v^3T due to the anharmonic oscillations of the atoms, and according to Sommerfeld (8) the effect of free electrons in the metal requires still another term of the form ET. The sum of these two terms may be represented with sufficient accuracy by a single term HT, since DC_v above is very small and nearly constant. The following equation therefore would seem to have a fairly sound theoretical basis:

$$C_p = C_\infty + HT + GC_v^3T - \frac{C_\infty\theta^2}{20T^2}\left(1 - \frac{\theta^2}{28T^2} + \frac{\theta^4}{910T^4}\right) \qquad (c)$$

Since the second and third terms of this equation are both small and the third term is nearly proportional to T, it is obviously impossible to evaluate the constants H and G separately with any degree of accuracy. It was decided, therefore, to adopt for G the value given above and solve for the three remaining constants. Using the same data as in the case of Equation (1) the following equation was obtained:

$$C_p = 0.3911 + 1.95 \times 10^{-5}T + 2.1 \times 10^{-4}C_v^3T - \frac{1998}{T^2}\left(1 - \frac{320^2}{28T^2} + \frac{320^4}{910T^4}\right) \qquad (2)$$

The values calculated from this equation are shown in Column 5 of Table IV. Although Equations (1) and (2) are seen to fit the experimental curve equally well, it would seem that the constants of the latter had decidedly more theoretical significance.

As a matter of interest, and in order to see how the various constants would be affected, Equation (3) was obtained in which $G'C_p^3T$ was used in place of GC_v^3T above.

$$C_p = .3938 + 1.34 \times 10^{-5}T + 2.0 \times 10^{-4}C_p^3T - \frac{2060}{T^2}\left(1 - \frac{323^2}{28T^2} + \frac{323^4}{910T^4}\right) \qquad (3)$$

The values calculated from this equation (Column 6, Table IV) also agree closely with the experimental points, but the slope of the experimental curve at 250° K. differs from that given by Equation (3) by more than the errors of measurement, which indicates that Equation (2) is preferable to Equation (3). For convenience the values for C_v calculated from data given in

TABLE IV

SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE K.

Temp., °K.	Experimental	Eq. (9J)	Eq. (1)	Eq. (2)	Eq. (3)	Value of C_v from Eq. (2)
200	0.3544	0.3525	0.3544	0.3544	0.3544	0.3493
225	0.3646	0.3639	0.3648	0.3647	0.36495	0.3586
250	0.3725	0.3724	0.37275	0.3728	0.3730	0.3658
275	0.3791	0.3790	0.3792	0.3791	0.3795	0.3712
300	0.3845	0.3845	0.38455	0.3845	0.38475	0.3756
325	0.3890	0.3890	0.3890	0.3890	0.3892	0.3792
350	0.3929	0.3929	0.3929	0.3929	0.3930	0.3822
375	0.3964	0.3963	0.3963	0.3962	0.3964	0.3845
400		0.3993	0.3993	0.3993	0.3993	0.3867
500		0.4093	0.4093	0.4092	0.4091	0.3930
600		0.4173	0.4173	0.4173	0.4171	0.3974
700		0.4244	0.4244	0.4243	0.4242	0.4007
800		0.4310	0.4310	0.4310	0.4310	0.4036

Equation (2) are given in the last column of Table IV. It should again be pointed out that between 270° and 800° *K.*, Column 3 represents the *experimental* results of the previous investigation (3).

Discussion of Equations

According to the Debye theory the value of C_∞ in these equations should equal $3R$ per mole or, in the case of copper, 0.3922 when the units are joules per gram. In none of the above equations does the constant term differ from this value by as much as 1% while in the case of Equation (2) the difference is less than 0.3%. The values of θ, the "characteristic temperature" of copper, obtained from Equations (1), (2) and (3) are 321°, 320°, and 323° *K.* respectively, which agree closely with the value 321° *K.* found from the T^3 Law. If the values of C_∞ or of θ in any one of these equations be slightly changed it is in general possible to adjust the other constants so that the new equation will fit the experimental curve about as well as the original, but the limits for such changes are decidedly less than 0.5%. Obviously greater accuracy and more certainty for these constants could be obtained by extending the range of temperature to still lower values, where the second and third terms of Equation (*c*) would be smaller and the terms containing θ would be larger.

A comparison of Equations (2) and (3) shows that the empirically determined value of H varies considerably, depending on the nature and magnitude of the term chosen for $C_p - C_v$, so that the value given in Equation (2) can be regarded only as a rough estimate which can be improved only by a more accurate knowledge of the term representing $C_p - C_v$. However, a term of this order of magnitude seems to be required theoretically and its inclusion in the Debye expansion is definitely necessary if the equation is to fit the experimental curve at high temperatures.

Equation (1) rather than the theoretically more exact Equation (2) has been selected for the abstract because it is more useful for purposes of calculation.

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SECULAR CHANGES OF THE MAGNETIC ELEMENTS, OTTAWA, 1500-1930[1]

By W. H. Herbert[2]

Abstract

In this paper is presented a table of the various elements of terrestrial magnetism at Ottawa from 1500 to 1930 and explains how the values were derived from old magnetic observations made in America, and not from theory. Among other points, it shows that though the total magnetic force has been declining at Ottawa for some time, yet the total magnetic force and the magnetic elements evidently go through cycles and none have apparently suffered permanent change during the time considered.

Original tables covering the secular change of declination for many places in Canada, 1750-1925, were compiled and published in 1926 by the Topographical Survey, Department of the Interior, in Bulletin 58: The March of the Compass in Canada. The main objects of those tables were to enable surveyors to retrace old lost land boundaries run by compass many years ago, and to afford this Survey a ready means of keeping up-to-date its more than 30,000 field measurements of declination made throughout the Dominion during the past half-century for the purpose of compiling every five years its declination chart of Canada—the only such chart published for this country.

In compiling those tables, the writer became interested in the wider question as to how far back the secular change of declination in Canada might be extended, and started to investigate the subject, soon discovering, however, that the time consumed in sifting over a large number of various old magnetic data might be better employed in investigating the secular changes of all the magnetic elements, instead of one only, especially since, as far as is known, the question had heretofore received but scant attention.

In this study the various old magnetic data had to be sorted out and co-ordinated for a large part of the eastern half of the western hemisphere in order to obtain consistent results. A short summary will serve to elucidate the methods used.

The values of declination were obtained by constructing charts for each half century using as a basis old charts such as that of Van Bemmelen, the Arcano del Mare and those in Hansteen's Magnetismus der Erde, modified and extended by early observations in Canada, such as those of Jacques Cartier, 1534, Master John Davis, 1585, Samuel de Champlain, 1610, Capt. Baffin, 1615, and others.

The change in horizontal force for each half-century interval was found by constructing, from the changes found in the declination, the horizontal magnetic field producing those changes.

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The values of inclination or dip were obtained by constructing charts for each century using as a basis the old charts of Hansteen, modified and extended by early observations such as those given in the Magnetismus der Erde, publications of the United States Coast and Geodetic Survey; and observations of Sir J. H. Lefroy, Sir George Back, Dr. Rae, Sir John Richardson, Thos. Simpson, Esq., Sir John Franklin, Sir E. W. Parry, Sir John Ross, and others.

While a single old original observation of declination or inclination may not be very accurate, average results derived from a considerable number obtained from many sources, such as were used in this investigation, are fairly good. It is conservatively estimated, from a long study of such data, that the maximum error of the old values of declination and inclination here quoted cannot exceed a quarter of one degree. Values quoted for 1850 and later are, of course, quite accurate.

The values of vertical and total force were derived from the values of horizontal force and inclination. The results are shown in Table I.

TABLE I

SECULAR CHANGES OF THE MAGNETIC ELEMENTS, MAJOR'S HILL PARK, OTTAWA
(Longitude, 75° 41′.8; Latitude, 45° 25′.7)

Date	D	I	H	V	F
1500	7° 00′ W.	76° 34′	0.1480	0.6200	0.6374
1550	10 30	76 55	.1400	.6025	.6186
1600	14 09	75 23	.1500	.5750	.5942
1650	16 29	73 07	.1700	.5600	.5852
1700	13 49	73 00	.1804	.5900	.6169
1750	9 50	74 27	.1730	.6220	.6456
1800	6 31	75 48	.1590	.6284	.6482
1850	8 46	76 40	.1450	.6118	.6288
1900	12 26	75 41	.1515	.5936	.6127
1930	14 28	75 31	.1470	.5691	.5878

D = declination or variation; I = inclination or dip; H, V and F = horizontal, vertical and total force, respectively, expressed in C.G.S. units.

This table shows that for the period of the 430 years considered, all the magnetic elements and the total force have changed with time, in apparent cycles having a period of about 325 years, but that apparently none have suffered any permanent change.

Attempts to express the apparent cycles of secular variations by harmonic analysis, exponential series or other mathematical expressions, have failed. It seems that there are many harmonics composing each secular variation, and that upon the harmonics are superimposed other fluctuations of temporary character or having a shorter period which will have to be eliminated before the true harmonic secular variation can be found. It is well known, by observation, that each station seems to have a different period for its secular variations, which differences are probably caused by temporary fluctuations

of shorter period. However, the reason for the difference in periods among stations is not yet solved. If more tables of secular variations, such as that quoted above, were available, many interesting questions in terrestrial magnetism, including the length of period for secular variations, might be nearer solution.

Note 1

Magnetic values observed at Major's Hill Park, Ottawa, by the Topographical Survey, Department of the Interior, 1930, were as follows: declination, 14° 28′ W.; inclination, 73° 31′; total force, 0.5878 c.g.s.

The horizontal and vertical forces for 1930 were computed from the total force and the inclination. The longitude and the latitude of the magnetic station were scaled off a map of one mile to one inch.

Note 2

In tracing out secular changes in inclination, use was also made of the fact that for stations having a high value of inclination, the inclination varies roughly inversely with the horizontal force.